Toward Ecological Holism:

Discerning Pattern, Process and Scale Across the Productivity Landscape

Angela M. Klock

A dissertation submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2021

Reading Committee:

Daniel Vogt, Co-chair

Kristiina Vogt

Heidi Gough

Program Authorized to Offer Degree:

School of Environmental and Forest Sciences

University of Washington

Abstract

Toward Ecological Holism:

Discerning Pattern, Process and Scale Across the Productivity Landscape

Angela M. Klock

Chair of the Supervisory Committee:

Associate Professor Daniel Vogt, Co-chair

Associate Professor Christian Torgersen, Co-chair

School of Environmental & Forest Sciences

Life is self-regulating because it is productive; capturing, storing, and passing energy through trophic compartments of the food web. Our current measurement frameworks for biological productivity rely heavily on measuring biomass in relation to first-order climatic factors, but this approach is insensitive to the mediating factors of genetics and adaptive capacity, metabolism and physiology, and ecological interactions and processes. Productivity is thus an emergent property of ecosystem

function, based on the collective metabolism of hosts and symbionts, interactions between species, and the flux of nutrients and energy across multiple levels of biological organization. The human enterprise is changing the structure and function of ecological systems around the world. As natural resources are degraded by pollution, nutrient loading, disturbance, and climate instability, ecosystems are being pushed past critical thresholds resulting in loss of biodiversity and declines in productivity. The internal processes of natural ecosystems are self-correcting, conditioned on the adaptive traits of organisms as they respond to local environmental conditions and each other. Foregoing chemoautotrophic ecosystems in extreme environments, oxygenic photosynthesis assembles the organic compounds upon which all terrestrial and aquatic ecosystems persist. Throughout the biosphere, symbiotic relationships mediate many physiological processes, from soil fungi providing nutrients for plant growth, to gut microbes metabolizing vitamins important to human health, all of which can change the metabolism, well-being, and productivity of hosts. I considered the elements of freshwater and forest ecosystems from which to reframe an understanding of ecological productivity, as trait-based and metabolism-driven, at multiple spatial scales of disturbance and across divergent ecological compartments. The work of this dissertation was to identify how shifts in small-scale physiological responses to disturbance pervade to large-scale ecological function. Understanding the internal processes of ecological stability requires integration of genetic traits and the phenotypic plasticity of those traits (i.e., adaptive capacity), within the broader context of the total environment. That is, there is a need to scale up and scale down. I integrated ecological productivity and disturbance at three spatio-ecological scales: (i) At the landscape level, I analyzed how disturbance influenced the distribution of bacteria with genetic traits for antibiotic resistance (a response to disturbance). I sampled 137 km of longitudinal river distance, from pristine headwaters to Superfund site near Seattle, Washington, USA. Antibiotic resistance was more prevalent in

tributaries and in moderate to highly developed stream banks, but resistance was not detected along river segments with intact riparian vegetation. (ii) To evaluate secondary productivity at the host-symbiont level, I collected bacteria that were carrying traits for antibiotic resistance from a polluted urban lake and introduced cultures of these organisms to microcosms of the keystone species, *Daphnia magna*. I measured the somatic growth, reproduction, and nutritional status of the *Daphnia* after 30-days. Results indicated secondary productivity was modulated by symbionts in different ways according to the primary ecology of the symbiont. (iii) I evaluated the primary productivity of forest ecosystems at the climatic-regional level with a calculated index, "ecosystem fit," (total net primary productivity/ theoretical maximum primary productivity) to estimate ecological resilience and scope-for-growth. Primary productivity was independent of tree leaf-traits, but phenology interacted divergently with climatic variables in extreme climates. Increases in biomass were associated with latitudinal gradients and seasonal forcing at the regional scale, and more closely associated with topographic and edaphic characteristics at the forest-stand scale. My analyses indicated that we can accurately predict which forests will be less resilient based on their current level of ecosystem fit and responsiveness across the full range of climatic regimes. With climate destabilization, we need to consider ecosystem function as emergent to all biotic and abiotic components that vary with scale. Each ecosystem is unique, and the most important variables will differ between them, and variable importance will change with scale, both within and between, different ecosystems. Ultimately, ecosystem properties are based on the collective metabolism of the dominant host species and all its symbionts, the collective phenotypic traits of the host-symbiont, and the genetic capacity for those traits to respond to environmental change.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

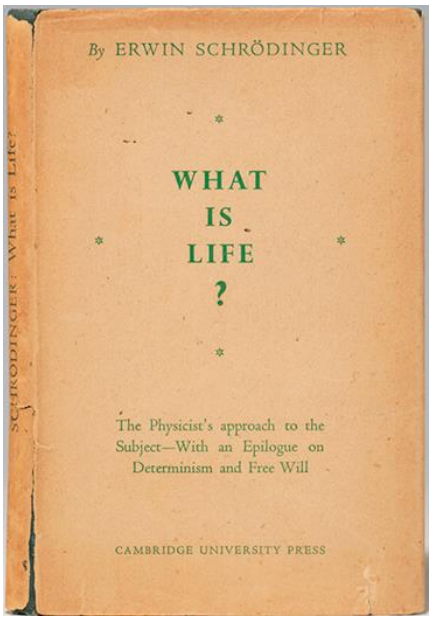
LIST OF TABLES

"A scientist is supposed to have a complete and thorough knowledge, at first hand, of some subjects and, therefore, is usually expected not to write on any topic of which he is not a master. This is regarded as a matter of *noblesse oblige*. For the present purpose I beg to renounce the *noblesse*, if any, and to be freed of the ensuing obligation...
I can see no other escape from this dilemma (lest our true
aim be lost for ever) than that some of us should venture to embark on a synthesis of facts and theories, albeit with second-hand and incomplete knowledge of some of them - and at the risk of making fools of ourselves."

Erwin Schrödinger

Preface to "What Is Life"

Dublin, September 1944



Chapter 1 : Toward a New View of Ecological Productivity

In 1859, Darwin wrote a best seller and laid down the central organizing principle of biology, evolution by natural selection. Many decades later Julian Huxley formally reconciled Darwinism and Mendelian genetics in his book, "Evolution: The Modern Synthesis" (Allen & Unwin, 1942). The work defined evolution as changes in allele frequencies for the first time and described phenotypic diversity as arising from random mutation and natural selection. Many of the barriers between various subdisciplines were removed and numerous important ecological theories that have advanced our understanding of how life works were formulated based on molecular data. Later in the 20th century, ecology migrated to a "gene-centric" phase fueled by the technologies of genomic sequencing, PCR, isolation of biological molecules and the "omics" (Vailati-Riboni et al., 2017). This culminated with the development of a new phylogeny based on ribosomal RNA and the elevation of the kingdom archaebacteria to domain (Woese & Fox, 1977; Woese et al., 1990). Generally, these approaches were empirically and metaphysically reductionist and stymied the holistic study of the life sciences.

"Molecular biology could read notes in the score,
but it couldn't hear the music" [Carl Woese, 2004]

Ecological research benefitted from rapid advances in computing power and new algorithms to model complex biogeochemical processes (Reed et al., 2014) but many studies suffered from design bias (Metcalfe et al., 2018) and field work was impacted by constraints external to the work (Marvin et al., 2014): economics (not enough money), convenience (not enough time), and lack of efficiency (not enough time or money) (Zvereva & Kozlov, 2021). The immersion in reductionist approaches and the technologies that fostered it have now run their course as we have "hit the wall

of biological complexity" (Woese, 2004). We now refer to kinesin proteins, a biological subunit of the cell, as "molecular machines" (Endow, 2003) and the twirling flagella of a bacterium as a "nano rotary motor" comprised of a universal joint, helical screw, rotor, a bushing and a drive shaft (Xue et al., 2015). There is value in thinking about life as a machine, we can devise energy budgets, understand the physics and mechanics of movement, and make predictions deterministically, but a machine cannot respond to its environment, cooperate, adapt, or self-organize, it is precisely the sum of its parts. In contrast, life is an emergent property of individual cells, working together, harnessing, and using energy, building biomass, and recycling limited resources, i.e., an organism.

1.1. Rise of the meta-organism and the holobiont

The evolution of eukaryotic organisms occurred after, and was embedded within, a microbial metabolic milieu such that multicellular organisms rely on their associated microbes for many aspects of their physiology including metabolism and immunological function. Technological advances in culture-independent analyses have identified the taxonomic and functional diversity of microorganisms associated with protists, plants, fungi, and animals (Douglas, 2014). Although driven by technology our conceptual understanding of what constitutes an individual organism is being challenged and a new level of biological individuality is under consideration, but the issue is not without controversy. Proponents argue that the high level of interdependence that exists between a host and its symbiotic microorganisms calls for a reframing of what constitutes an individual unit of selection in evolution. Opponents do not dispute the concept of obligate symbioses, i.e., mitochondria and chloroplasts in eukaryotes, nor that bacteria are sometimes integrated closely enough to constitute a unique biological unit (e.g., *Buchnera aphidicola* in aphids, *Wolbachia* spp. in insects) but they draw the line at wide-scale functional integration for all host-symbiont relationships (Suárez & Triviño, 2019).

Symbiosis constitutes a dynamic gradient of conditions ranging from antagonistic to mutualistic (Dimijian, 2000) and the host-symbiont interaction a continuum from transient to obligate. Symbioses are not limited to eukaryotic hosts with prokaryotic symbionts (e.g., coral-algae, plants-mycorrhizae), and further confounding the issue are lichens, a "composite" organism formed by a close association between a fungus and algae and/or cyanobacteria, with distinct properties of each. Recent research on the lichen microbiome has revealed host-specific bacterial communities that contribute functionally to the entire lichen, a multi-species symbioses that is sensitive to environmental and ecological factors (Aschenbrenner et al., 2016). Multi-cellular organisms are not a single individual: we must account for the community of organisms in and on their bodies when analyzing ecological systems and interpreting their function at higher levels of organization.

"Much advance in evolution is due to the establishment of consortia between two organisms with entirely different genomes. Ecologists have barely begun to describe these interactions." [Ernst Mayr (1904-2005)]

Richard Dawkins proposed that natural selection would favor selective altruism, *aka* cooperation (Dawkins, 1976; Dawkins, 1982). The first ancient cells began cooperating when they formed simple by-product food exchanges (Stewart, 2019) and evidence supports cooperation between molecules that predated cells (Attwater & Holliger, 2012; Higgs & Lehman, 2015). These relationships grew in complexity to obligate mutualisms with the endosymbiotic origin of the eukaryotic cell almost two billion years later (Sagan [Margulis], 1967; Gray, 2017). This effectively makes eukaryotes genetic chimeras. Margulis believed Darwin's proposition of natural selection as the primary driver of evolution was incomplete, "Life did not take over the globe by combat, but by

networking" (i.e., via cooperation, interaction, and mutual dependence) (Margulis & Sagan, 1996). The term "metaorganism" better describes the *functional relationship* between a host and its associated microbiome (Jaspers et al., 2019).

Ecosystems and the organisms that comprise them are coupled by microbial biogeochemical cycles of nutrients and energy vital to maintaining a habitable biosphere. This is well established ecological theory, but technology has provided new insights to the ubiquity, diversity, and importance of prokaryotes in every aspect of metazoan biology. A new philosophy of microbiology (O'Malley, 2014) has emerged to rehabilitate our notion of microbes, one that embraces a holistic view of biological complexity that is consistent with the methods of the past; observation (e.g., Darwin), theoretical (e.g., Hutchinson), applied (e.g., Woese), and metagenomic (e.g., Handelson), and one open to technological advances of the future. O'Malley wants us to place microbiology front-and-center in all aspects of biological inquiry due to their ubiquitous metabolic interactions with all other life forms; giving precedence to "higher-level entities over lower-level units." She states, "evolution simply is microbial; microbes have been around much longer, most evolution on earth has been microbial evolution, all novel metabolic capacities evolved in microbes, ecology (macrobes depend on microbes and live commensally with them), and microbial biogeochemical processes make life possible."

"This is truly the 'age of bacteria' – as it was in the beginning, is now and ever shall be."

[Stephan Jay Gould (1994)]

Clearly a paradigm shift in the perception of microbes is happening in the public sphere and "microbiome" is now colloquial. Many ecologists view the traits of multi-cellular organisms as

emergent to the complex interactions between the organism itself and the microbes it hosts. These interactions result from the *combined expression* of a host and the hosts' microbial genomes, that is the "holobiont" (Zilber-Rosenberg & Rosenberg, 2008; Bordenstein & Theis, 2015). The holobiont is a higher-order organism with properties reflecting the collective metabolism of the host and all of its symbiotic microflora (Zilber-Rosenberg & Rosenberg, 2008).

"In nature, nothing exists alone." [Rachel Carson (Silent Spring, 1962)]

The term holobiont accounts for the entirety of the microbial genetic diversity associated with a host whether it provides a known functional benefit or not (Jaspers et al., 2019). This has produced a bank of genetic information that may inform future functional roles attributable to symbionts. There is debate as to treat holobionts as individuals or as emergent individual units of selection. Ignoring the semantic argument, the function of the holobiont is already implicit in ecology and metrics of productivity but how do we account for the contributions and influence of each? The problem is twofold: (i) we lack information about natural holobiont community diversity and function; and (ii) baseline productivity indices from pristine environments are unavailable due to wide-spread disturbance to ecosystems. This will make it difficult to assess the true impact of ongoing environmental change to holobiont assemblages and ecological function in many biomes.

"I speculate that we shall come to accept the more radical idea that each one of our genes is a symbiotic unit. We are gigantic colonies of symbiotic genes."
[Richard Dawkins (The Selfish Gene)]

The "hologenome theory" of evolution proposes that the holobiont is a unit of natural selection, due to several general principles: i) symbiotic relationships are ubiquitous in nature; ii) symbionts are transferred intergenerationally; iii) interactions between host and symbiont influence fitness; iv) changes can occur selectively in host and symbiont genomes, and v) the evolvability of the holobiont is enhanced because symbionts respond quicker to environmental cues (Zilber-Rosenberg & Rosenberg, 2008). Over billions of years of evolution, the holobiont has struck the cooperative balance necessary to meet the environmental and ecological conditions in which it evolved. What remains unclear is how the holobiont will respond to climate change. The holobiont response will depend on the conditions it evolved under and whether and how those conditions imbued the capacity for ecological flexibility into the genome. The holobionts with symbiotic communities that can respond quickly to the environment may fair better (Zilber-Rosenberg & Rosenberg, 2008), whereas holobionts with highly specialized or strong obligate symbiotic relationships may be incapable of symbiotic realignment putting the species at higher risk of extinction (Bennett & Moran, 2015). The success stories will probably be those holobionts with responsive and effective microbial "first responders" which have enabled niche expansion for their hosts in the past.

Species that evolved in highly variable environments may host the most flexible symbionts, whereas predictable environments such as tropical forests (temperature, precipitation), coral reefs (temperature, pH), and old growth forests (ecology) may have well-established intransient symbionts. Strong seasonal forcing may also foster strong symbiotic relationships incapable of responding quickly to earlier spring temperatures or warmer winters. Specialized adaptation to environmental conditions, inter-specific dependencies, or strong seasonal forcing often transitions into more narrow ecological niches. A narrow niche offers advantages in highly diverse and

competitive ecosystems such as tropical forests but can also be a nail in the extinction coffin with accelerated environmental change such as shrinking home ranges and loss of adaptive capacity (Raia et al., 2016).

1.2. The ultimate regress to physics

Life is thermodynamically driven (England, 2013). We can conceptualize this as living systems forming small whirlpools of complexity and structure in the stream of solar energy as it flows out into the universe toward thermodynamical equilibrium, that is, maximum entropy. Life does not violate the Second Law of Thermodynamics because life dissipates heat and increases entropy in its immediate surroundings. For example, trees absorb high energy sunlight to reduce carbon from CO_2 into molecules of sugar releasing heat as they grow. Individual cells are highly organized, organisms are composed of cooperating communities of cells, and populations are composed of interacting communities of organisms, each an isolated, although larger, eddy in the flow. Life is bound by the laws of physics and has evolved to optimize resources by partitioning them to dissipate the most heat, most efficiently, but do not take my word for it!

"All science is either physics or stamp collecting." [Ernest Rutherford, 1939]

To effectively increase entropy, life builds more and more complexity into bodies and develops highly complex relationships between bodies to further partition a resource. Raymond Lindeman (1915-1945) wrote a set of rules by which energy is transferred within ecosystems known as the 10% rule. Lindeman's proposal was advocated by the "father of modern ecology," G. Evelyn Hutchinson, and the papers were published in *Ecology* shortly after Lindeman's untimely death in

1942 (Lindeman, 1942a; 1942b). In these papers he laid out the concept of the inefficiencies inherent in biological systems as energy accumulates and is then transferred via herbivory, predation and decomposition to organisms occupying different trophic compartments (levels) in the ecosystem. Energy transformation is never 100% efficient, and in freshwater lakes, Lindeman determined about 90% of the free energy available in one trophic compartment was lost and unavailable to the next higher trophic compartment.

Microbes and insects frequently display efficiencies greater than 10% but high efficiency often comes with considerable tradeoffs (Sanders et al., 2016). For example, 3rd and 4th-level hyperparasitoid relationships are tenuous, and the loss of a single intermediary host can result in the collapse of entire chain of host-parasite symbioses (Harvey et al., 2003). Food chain length is also a hierarchical ecological principle first described by the "father of animal ecology" Charles Elton in 1927, where larger ecosystems are positively correlated with longer food chains and higher resilience and shorter food chains are unlikely to persist in disturbed habitats (Elton, 1927; Doi & Hillebrand, 2019).

> *"Food is the burning question in animal society, and the whole structure and activities of the community are dependent upon questions of food-supply."* [Charles Elton (1960)]

Hutchinson and Lindeman advanced the field of ecology from a formerly descriptive "soft" science (Pigliucci, 2002) to a theoretical "hard" science with the integration of mathematical modeling, meticulous study design, and incorporation of biogeochemistry to understand ecological processes. Lindeman also proposed the bio-ecological community concept where all organisms

associated with a particular zone form a "primary ecological unit." For example, a forest is a primary ecological unit where the ecology of each community within the forest such as soils or epiphytes are dependent upon the entire forest food web. The forest is in-turn dependent upon the flux of energy and nutrients from the wider landscape, and so it goes.

Ecological units are not defined by size, but rather they are any system that is self-sustaining and self-regulating, and as an ecological unit is composed of disparate components, we need to place it within the broader context of the total environment to understand its internal processes. Therefore, the science demands scaling up and scaling down. For example, to scale down in a terrestrial system, we may consider local topography, soil structure and soil microbial community composition, quantity of leaf litter, decomposition rates, or the availability of inorganic nutrients. In a lake ecosystem, the sedimentation rate of particles and timing of turnover would be important, or in a river, the flow rate and metabolism of epilithic biofilms, etc. Features and processes to consider are more specific with ever-finer tessellations of the ecological units and ultimately regress to the cellular level as the physical properties of the system and nutrient stoichiometry driven by the community composition of heterotrophic bacteria have their say.

"If I could do it all over again, and relive my vision in the twenty-first century, I would be a microbial ecologist. Ten billion bacteria live in a gram of ordinary soil, a mere pinch held between thumb and forefinger. They represent thousands of species, almost none of which are known to science. Into that world I would go with the aid of modern microscopy and molecular analysis. I would cut my way through clonal forests sprawled across grains of sand, travel in an imagined submarine through drops of water proportionately the size of lakes, and track predators and prey in order to discover new life ways and alien food webs". [Edward O. Wilson (1994)]

A primary small-scale driver of primary productivity is phosphorus and it is frequently the most limiting nutrient in natural forests (Ludovick Achat et al., 2016). After accounting for exogenous sources, the rate of geological decomposition determines the amount of phosphorus available for primary production in terrestrial ecosystems. As phosphorus is essential in nucleic acids and cell membranes, and decomposes slowly from the parent material, phosphorus yields a very strong bottom-up constraint. Conversely, phosphorus is commonly overloaded in freshwater (Mekonnen & Hoekstra, 2017) and increases primary productivity. In moderate amounts, some systems benefit, but generally the consequences are detrimental resulting in eutrophication and anoxic conditions that kill fish, depressing biodiversity and can push ecosystems over tipping points.

To scale up, we consider the surrounding landscape, watershed, region, biome, and climatic zone. The tessellation of ever-larger units influences more and more generally as spatial scale increases. At the largest scale, climatic variables are first-order controls over primary production in forests (Toledo et al., 2011), and CO_2, light, and nutrients play a significant role in marine systems with secondary factors of depth, temperature, and wind also impacting productivity (Choi et al., 2015). With freshwater ecosystems, light, pH, and nutrient ratios strongly control phytoplankton productivity (Jia et al., 2020). Variation of the values of the first-order drivers create a strong top-down influence on community composition, i.e., the types of primary producers, and their phenotypic traits and life history.

"A single tree by itself is dependent upon all the adverse chances of shifting circumstances...but in nature the normal way in which trees flourish is by their association in a forest...a forest is the triumph of the organisation of mutually dependent species."

[Alfred North Whitehead (1927)]

Phenotypic traits of the predominant primary producer are the strongest determinant of ecosystem productivity and represents an "ecosystem property." Therefore, "ecosystem properties" are a functional unit of natural selection and one which can be readily assessed (Violle et al., (2007). A high-level forest ecosystem property is tree leaf phenology. Phenology is not determinative of productivity, but the major cues that direct deciduous phenology in high latitudes are temperature and photoperiod. These variables frequently interact so the response is often non-linear, and productivity is more difficult to predict. Alternatively, if phenotypic traits are predominately driven by a single variable like temperature or precipitation predictions are more reliable (Flynn & Wolkovich, 2018). In equatorial latitudes low precipitation is the dominant cue for leaf senescence and therefore predicting productivity in tropical forests is potentially more robust.

1.3. An ecosystem is defined by its metabolism

The entropy of the universe (a closed system) is being helped along by photosynthesis as the process eventually transforms all the high energy sunlight collected into low energy heat through cellular respiration of plants and animals. Trees remain highly structured and complex and avoid maximum entropy...death, by scooping up free energy from the environment and building tissue via oxygenic photosynthesis, thus keeping the earth's atmosphere in disequilibrium (Krissansen-Totton et al., 2018).

"Life seems to be orderly and lawful behavior of matter, not based exclusively on its tendency to go over from order to disorder, but based partly on existing order that is kept up" [Erwin Schrödinger, 1943]

That is, life makes stuff! Three types of ecological fitness have been proposed: the ability to compete (survivability), the ability to cooperate (symbiosis), and the ability to *construct* (metabolism) (Peacock, 2011). An organism that can increase the energy-circulating capacity within its ecosystem (biomass) will have an increased probably of survival because it has increased the carrying capacity of its ecosystem. Logically, trees have high constructive fit because they are large, long-lived, and are efficient at increasing biomass (Peacock, 2011). Marine phytoplankton are tiny but are responsible for more than half of global primary production, with higher carbon fixation rates and higher biomass productivity than any other photosynthetic organisms (Kwiatkowski et al., 2018). How can this be?

"I had no idea that animals of such low organization could develop such extremely beautiful structures"

[Darwin replying to Ernst Heckle on radiolarians, 1864]

There are many contrasts that can be drawn between aquatic and terrestrial food-webs, but the total productivity (primary and secondary) of an ecosystem is based on the growth rate, size, and nutritional quality of its autotrophs. Autotrophs come in many different flavors but all function with the same photosynthetic machinery, i.e., chloroplasts. Algae are small and easy to digest because they lack the complex and metabolically challenging structural tissues required by terrestrial primary producers. This means they can achieve faster growth rates and provide higher nutrition to herbivores than terrestrial primary producers (Shurin et al., 2006). The issue of scale imposes strong control over how productivity influences other trophic compartments as singled-celled phytoplankton are the dominate primary producers in aquatic systems and multi-cellular plants are the dominate primary producers in terrestrial systems (Lindeman, 1942b).

Algae species differ in size by several orders of magnitude (0.2-200+ μm) and size has strong selective power on the community composition of grazers. This selection does not go down without a fight, however, as an "evolutionary arms race" is ongoing between phytoplankton (with their defenses against grazers) versus their predators (who develop ways to thwart the phytoplankton defenses) (Irigoien et al., 2005). This constrains primary productivity as energy has be to diverted to defenses against herbivory (Smetacek, 2001), resulting in different characteristics of the dominant primary producers with knock-on effects to food-web structure (food chain length, specialization, nutrition) (Shurin et al., 2006). The arms race plays out in terrestrial ecosystems in the same way, a series of one-upmanship's between producers and consumers with plants hosting thorns, producing toxins, being unpalatable or poor-quality food to discourage herbivory.

The notion can be expanded to gauging the performance of constructive fitness by comparing the ratio of the measured productivity to the productivity that is theoretically possible based on local conditions without constraints, an "ecosystem fit." Ecosystem fit is a proxy for ecological resilience as it is significantly correlated with productivity ($p < 0.05$). Productivity generally scales with biodiversity (Tilman et al., 2012), but the strength of the correlation between them is highly variable in different forest ecosystems (Liang et al., 2016). There is considerable variability of small-scale conditions (micro-climate, topography, soils), and this produces a wide range of productivity and ecosystem fit values in each climatic zone. These productivity responses are a function of different combinations of site-level conditions and interactions between environmental conditions and the collective metabolism of the holobiont.

Metabolism defines life at all levels of biological organization and controls the movement of carbon through the biosphere, in short, metabolism makes bodies. At any level of biological organization when the rate of carbon uptake (anabolism) is equal to the rate of carbon loss

(catabolism), the system is said to be in steady-state. The rate of carbon fixation *is* gross primary productivity in autotrophic holobionts and the residence time of carbon (turnover) can vary by many orders of magnitude depending on the species involved, i.e., phytoplankton versus redwoods. The average residence time of carbon in an ecosystem is equal to total carbon biomass divided by the uptake rate. Much of the variation in the carbon flux can be described by scaling theory and organismal allometry (Marquet et al., 2005) but not all.

Natural ecosystems are generally nutrient limited (Elser et al., 2007) and this is reflected in the nutritional value of the primary producers. Dependent upon the quality of food consumers make "choices" about how to allocate resources between maintenance, growth, and reproduction. To reproduce with resource limitations organisms can either maintain the same allocation proportions or vary their phenotypic strategy by shifting resources to one trait over another (Ng'oma et al., 2017). In nature, organisms respond to variability in resources dynamically over space and time. Therefore, evolution should favor allocation strategies that can respond rapidly to changes in resource levels (King & Roff, 2010). Holobionts will exhibit different trade-off strategies in their resource allocation ratios depending on the predictability of resource levels over evolutionary time scales, their life-history traits, and the phenotypic plasticity of those traits.

1.4. Patterns, scale, and scaling

The science of ecology is about resolving the problems of scale and pattern where the ability to make predictions demand knowledge of the underlying mechanisms (Levin, 1992). Biological systems are complex but many aspects of metabolism, biomass, and species abundance can be described by simple scaling relationships across taxa and habitat (Marquet et al., 2005; Locey & Lennon, 2016; Jenkins & Pierce, 2017) or by trophic compartment where organisms from unrelated taxa comingle (Schramski et al., 2015). For example, the latitudinal diversity gradient of species is

one of the best-known concepts in ecology, having widespread common knowledge for centuries. Although biodiversity in the tropics and biological paucity at the poles is obvious, the mechanisms behind this phenomenon remain unclear today with dozens of theories proposed in the literature (Hillebrand, 2004). There are several generalities in regard to latitude, such as Bergman's Rule, animals get larger toward the poles (*circa* 1847), Allen's Rule, cold-adapted animals have shorter limbs and appendages than warm-adapted animals (*circa* 1877), low latitudes are correlated with forest species richness (Zhang et al., 2006), and high latitudes are correlated with better photosynthetic conversion efficiency (carbon fixation to biomass) (Kaluthota et al., 2015). Photosynthetic conversion efficiency is temperature dependent, and although it is a parameter of photosynthesis, primary productivity is only moderately associated with latitude. Primary producers in all climates have compensatory mechanisms to boost productivity under less favorable conditions such as low temperatures, high elevation, low precipitation, or a short growing season. In addition to compensating for harsh conditions, primary productivity is better predicted by different combinations of temperature, and precipitation at the biome level and different combinations of temperature, precipitation, and soil conditions at the site-level.

1.5. *Daphnia* as a model organism for secondary productivity

Daphnia species occur in standing freshwater systems on every continent where they are keystone species in aquatic food-webs. In the wild, when conditions are good *Daphnia* reproduce by serial parthenogenesis and populations can expand rapidly. Their short generation time and labile symbiotic community make this genus a valuable model organism for a variety host-symbiont experiments (Sison-Mangus et al., 2015).

Daphnia display a range of phenotypically plastic morphological, physiological, and behavioral adaptations that has the capacity to influence their interactions with predators and

increase individual and population fitness (Weiss, 2019). Phenotypic plasticity can be induced by environmental conditions (nutrients, temperature) or ecological interactions (competition, predators) (Weiss, 2019). The mechanisms of phenotypic plasticity are being explored (Boersma et al., 1998; Christjani et al., 2016) with proteomic technology to determine how *Daphnia* trait variation and trait plasticity are linked to environmental conditions (Tams et al., 2020).

Morphological changes in response to predator cues are common among many different species of *Daphnia* and include growing larger (size refugia) and producing spines (increasing handling time of predators) (Weiss et al., 2012) (Fig. 1-1), or exhibiting changes in life history by shifting resources from somatic growth to sexual maturity at a smaller size (Brett, 1992). *Daphnia* display behavioral plasticity (vertical migration, swimming speed) in response to the presence of predators, and body-size plasticity in response to food quality. Prey body size drives selection in two dimensions, the predator cohort due to handling time constraints, and the plankton community composition due to feeding (Compte et al., 2009).

Daphnia feed on bacteria and the proportion of bacteria in the diet varies by lake trophic status and season (Taipale et al., 2008). The efficiency with which *Daphnia* filter bacteria from the water column depends on the mesh-size of their filter combs, and although mesh size is species-specific and independent of body size, this trait is also highly plastic (Bednarska & Dawidowicz, 2007). These examples of *Daphnia* trait responses are direct links of gene function to ecological community structure and functional attributes via both top-down and bottom-up mechanisms. Epigenetic marks have been detected on the *Daphnia* genome (Vandegehuchte et al., 2010) indicating possible epigenetic control of trait expression and adaptation to environmental challenges (Harris et al., 2012).

Daphnia require a bacterial microbiome for survival, without one they are smaller, less fecund and have higher mortality (Sison-Mangus et al., 2015). Environmental contamination by antibiotics and antibiotic resistance determinants changes food incorporation rates and assimilation efficiencies in *Daphnia* (Wollenberger et al., 2000). Exposure to antibiotics selectively kill certain types of bacteria resulting in changes to the *Daphnia* microbiome which compromises nutrition and results in slower growth (Cooper & Cressler, 2020). These effects may not be unique for antibiotics and may apply to other environmental pollutants (Gorokhova et al., 2015). The widespread contamination of urban surface waters poses health risks for humans, animals, and the environment. Due to the flux of nutrients between aquatic and adjoining terrestrial environments, we should expect that there will be knock-on effects to terrestrial-associated food webs from compromised production in aquatic systems.

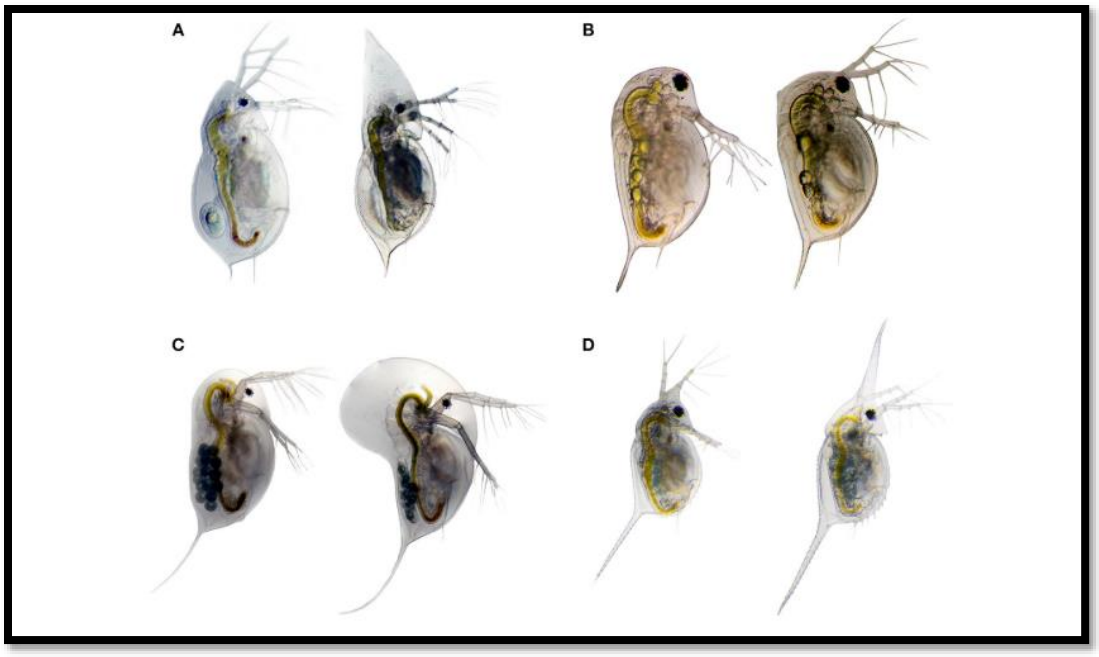


Figure 1-1: Inducible morphological defenses are manifold in the genus *Daphnia*. The listed examples show: (A) helmet expression in *D. cucullata*; (B) neckteeth expression in *D. pulex*; (C) crest expression in *D. longicephala*; and (D) head- and tail-spine formation in *D. lumholtzi*. Undefended morphotypes are displayed on the left and defended morphotypes on the right. Images by Becker & Weiss. Used with permission (Weiss, 2019).

1.6. Enteric bacteria and antibiotics in the environment

The water column and sediments of urban surface waters are spatially heterogeneous due to natural and anthropogenic factors. Many systems are pervasively contaminated with anthropic sources of bacteria and other pollutants including antibiotics, but the spatial and functional diversity of bacterial communities in water and sediments remain unclear. Urban rivers provide unique opportunities for the amplification, diversification, and circulation of bacteria and bacterial traits to-and-from human, animal, and environmental ecologies. Bacterial communities fluctuate substantially between environmental sites but the Gammaproteobacteria are significantly enriched in urban rivers implying origins arising from human-based activities (Ge et al., 2021). The Gammaproteobacteria include many medically, ecologically, and scientifically important groups of Gram-negative bacteria including many pathogens that harbor genes for antibiotic resistance. This group includes common enteric coliforms including *Escherichia coli* which regulatory agencies use as an index of fecal contamination.

1.7. The "landscape" as metaphor

The "epigenetic landscape" is a conceptual model Conrad H. Waddington presented in his text "The Strategy of the Genes" (Allen & Unwin, 1957). This framework describes the developmental pathways a cell takes leading to final type, or how extracellular cues regulate gene expression during embryonic cellular differentiation (Waddington, 1940; Waddington, 1957). Epigenetic marks on the genome modulate gene activity by turning the expression of genes "on" or "off" at critical times during development which ultimately determines the phenotype of the organism. Waddington envisioned the epigenetic landscape as a series of valleys and ridges through which a cell may travel on its journey to a final fixed cell type. The idea of equilibrium was an

important concept of the epigenetic landscape. Although the ball tends toward the center of a channel, the overall equilibrium tends downward, i.e., long-term trajectory allowing for short-term variability. These are epigenetic factors that can change cell fate along the way.

Figure 1-2: Tattoo of Waddington's conceptual model of the epigenetic landscape on a true believer.

Whereas Waddington's model centered on embryonic development and cell fate, the concept can be applied to trait plasticity and its influence on ecological productivity. Continuing the metaphor, the ability of the ball to move within its lane as it rolls downhill is the level of plasticity permitted by the genome, i.e., the amount of variability inherent within the genome (including epigenetic effects). The epigenetic component of plasticity is less well understood but there is evidence that the environment can induce epigenetic mechanisms, e.g., the development of prickly

leaves in response to herbivory (Herrera & Bazaga, 2013). A highly channelized (genetically constrained) species has limited capacity for adaptive plasticity.

In addition to genetic limitations, there may be thresholds in environmental conditions where the phenotypic response stabilizes or is punctuated up or down. For example, after a wildfire, gaps open in the canopy and shade-intolerant trees colonize the open space quicker than shade-tolerant species, the order of succession. Conversely, adaptive plasticity presenting at different stages of growth may confer advantages such as shade-tolerant seedlings being able to out-compete shade-intolerant seedlings in the understory (Gratani, 2014).

1.8. The "productivity landscape"

The productivity landscape is a conceptual model of primary and secondary productivity, trophic compartments, ecosystem properties, and ecological resilience (Fig. 1-1). Bottom-up controls link productivity to biogeochemistry (soil and symbiont compartments) and top-down controls link productivity to climate (temperature and precipitation). A "middle-out" approach accounts for having specific knowledge about a target organism or ecosystem while coupling the bottom-up and top-down controls to the response, always remembering to not get bogged down in too much detail or miss important feedbacks by over-simplification of a process. Predictive models of productivity responses must be balanced to provide a framework to understanding the potential impact of climate destabilization.

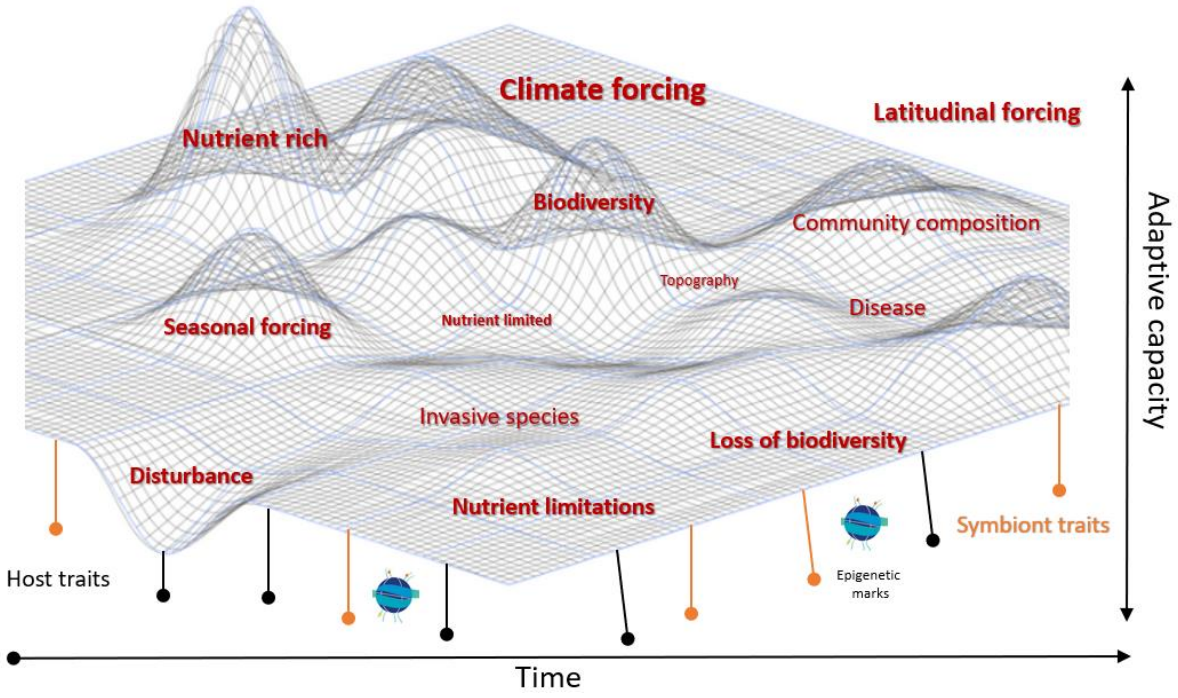


Figure 1-3: Conceptual model of the "productivity landscape." Underpinned by the genetic traits and the adaptive capacity of the genetic traits of the host and all its associated symbionts, the "holobiont." Multiple environmental and ecological factors will push and pull on productivity metrics over space and time.

Primary production in terrestrial and aquatic habitats is highly variable, but the total biomass produced is similar between them (Geider et al., 2001). Secondary production has been proposed as a more comprehensive metric of ecosystem assessment as it reflects multiple ecosystem properties such as individual growth, reproduction, survivorship, and species interactions (Dolbeth et al., 2012). When comparing different forest ecosystems, primary productivity is not informative to the total level of secondary productivity (Miner et al., 2012). If herbivory removes a large percentage of biomass this leaves a small percentage of the carbon fixed by producers sequestered in leaves, stems, and roots, i.e., grasslands. This means a strong top-down control is being exerted by herbivores. When this occurs, the recycling of carbon and remineralization of nutrients available to primary

producers is faster. This holds true in terrestrial habitats, but aquatic habitats can behave differently. Marine algae production can support large numbers of grazers and not sequester much carbon in photosynthetic biomass as it is grazed away as quickly as it is produced, e.g., coral reefs. Therefore, estimates of secondary productivity represent the functional responses of entire communities after important ecological interactions have taken place.

Generally, secondary productivity in aquatic and terrestrial ecosystems is about an order of magnitude less than the primary productivity (i.e., the 10% rule) but there are caveats. Food webs based on trees will have more herbivores in a given area than trees, whereas food webs based on phytoplankton may have inverted pyramids of biomass, as algae are microscopic with fast generation times, are highly productive, and can maintain a large proportion of animal biomass (phytoplankton→ krill→ blue whale) (Brown et al., 2004). This mechanism functions similarly with total decomposition, i.e., the amount of detritus consumed by decomposers and detritivores. This has important implications for mycorrhizal symbionts as the decomposition rate is indicative of the level of below ground productivity because only a small proportion of the carbon metabolized by decomposers ends up as decomposer biomass. When decomposition rates are high the total residence time of carbon and nutrients is short and less carbon is stored in detritus or as below ground productivity, as occurs in tropical forests.

Ecological boundaries are fluid, and the processes of adaptation are based on disequilibrium with oscillations of dynamic systems being difficult to interpret and predict. The problem lies in our paradigms which are mostly conceived and understood at human scales. Typically, scientific research tends to reinforce existing community standards and the underling paradigm, and experimental design tends to align with the existing paradigm. Thus, well-established methodologies are recalcitrant to change and application at larger and smaller scales is tricky. As we use models to make

predictions, we must tune our model parameters and change our explanatory variables to what is relevant at the scale of interest, and to the most meaningful ecological context of the system, while accounting for variability of each over time and space. This is a complex puzzle to solve.

I considered three systems at three spatio-ecological scales:

1) At the watershed scale, I identified bacteria in a 4th-order urban river to test whether environmental conditions determine bacterial trait prevalence. I evaluated how land use changed the distribution of traits as a function of broad scale land- and river scape differences. I found significant variation in bacteria type and resistance traits between segments of the river with very good to very poor water quality, level of development, and environmental conditions. Differences between the forested headwaters and the highly contaminated estuarine habitats support an alternative, ecological perspective to suggest that bacterial functional traits arising from different evolutionary backgrounds can exhibit different tradeoffs in response to environmental stressors.
2) At the organismal scale, I applied the knowledge of the extent and prevalence of anthropic bacterial pollution to a model organism to understand the potential of how these common bacteria pollutants may influence aquatic food webs. Utilizing an established experimental design to control for environmental and genetic traits, I compared physiological functional traits relating to growth, fecundity, and fatty acid biosynthesis in *D. magna*. I found that productivity selectively differed among the bacterial treatments with higher productivity being associated with wild bacterial strains regardless of their antibiotic resistance profiles, and lower fecundity, survivorship, and poorer nutritional value with the existing laboratory culture-based epibionts and a clinical strain of *E. coli*.

3) And at the global scale, I developed a predictive model of net primary productivity and calculated the ecosystem fit of forests in boreal, temperate, and tropical climates. Forest ecosystems are second only to the oceans as a climate stabilizing force on earth, provide habitat for diverse wildlife, function to recharge aquifers, and provide innumerable societal resources and benefits. Unlike highly dynamic ecosystems such as rivers with high disturbance regimes and rapid change in habitat structure, I identified thresholds where productivity and forest type could be accurately predicted. I used machine learning algorithms to analyze the climatic and edaphic conditions to identify environmental thresholds, the safe operating space for growth, and reaction norms, i.e., the value of a phenotypic trait (productivity) for a genotype (phenology) along gradients of the most important environmental factors.

References

Aschenbrenner, I. A., Cernava, T., Berg, G., & Grube, M. (2016). Understanding microbial multi-species symbioses. *Frontiers in Microbiology*, *7*(FEB), 1–9. https://doi.org/10.3389/fmicb.2016.00180

Attwater, J., & Holliger, P. (2012). Origins of life: The cooperative gene. *Nature, 491*(7422), 48–49. https://doi.org/10.1038/nature11635

Bednarska, A., & Dawidowicz, P. (2007). Change in filter-screen morphology and depth selection: Uncoupled responses of *Daphnia* to the presence of filamentous cyanobacteria. *Limnol. Oceanogr., 52*(6), 2358–2363.

Bennett, G. M., & Moran, N. A. (2015). Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences of the United States of America, 112*(33), 10169–10176. https://doi.org/10.1073/pnas.1421388112

Boersma, M., Spaak, P., & De Meester, L. (1998). Predator-Mediated Plasticity in Morphology, Life History, and Behavior of *Daphnia*: The Uncoupling of Responses. *The American Naturalist, 152*(2).

Bordenstein, S. R., Theis, K. R. (2015) Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLoS Biol 13*(8): e1002226. https://doi.org/10.1371/journal.pbio.1002226

Brett, T. (1992). Chaoborus and fish-mediated influences on *Daphnia longispina* population structure, dynamics and life history strategies. *Oecologia, 89*, 69–77.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward A Metabolic Theory of Ecology. *Ecology, 85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Carson, R. (2000). Silent Spring. Penguin Classics

Choi, J. K., Noh, J. H., Orlova, T., Park, M. O., Lee, S. H., Park, Y. J., Son, S., Stonik, I., & Choi, D. H. (2015). Phytoplankton and primary production. *Oceanography of the East Sea (Japan Sea),* 217–245. https://doi.org/10.1007/978-3-319-22720-7_10

Christjani, M., Fink, P., & von Elert, E. (2016). Phenotypic plasticity in three *Daphnia* genotypes in response to predator kairomone: evidence for an involvement of chitin deacetylases. *The Company of Biologists, 219*, 1697–1704. https://doi.org/10.1242/jeb.133504

Compte, J., Brucet, S., Gasco, S., Boix, D., Sala, J., Lopez-Flores, R., & Quintana, X. D. (2009). Impact of different developmental stages of *Daphnia magna* (Straus) on the plankton community under different trophic conditions. *Hydrobiologia, 635,* 45–56. https://doi.org/10.1007/s10750-009-9860-3

Cooper, R. O., & Cressler, C. E. (2020). Characterization of key bacterial species in the *Daphnia magna* microbiota using shotgun metagenomics. *Scientific Reports, 10*(1), 1–11. https://doi.org/10.1038/s41598-019-57367-x

Dawkins, R. (1976). The Selfish Gene. Oxford University Press.

Dawkins, R. (1982). The Extended Phenotype. Oxford University Press, Oxford.

Dimijian, G. G. (2000). Evolving together: the biology of symbiosis, part 1. *Baylor University Medical Center Proceedings, 13,* 217–226.

Doi, H., & Hillebrand, H. (2019). Historical contingency and productivity effects on food-chain length. Communications *Biology, 2*(1). https://doi.org/10.1038/s42003-019-0287-8

Dolbeth, M., Cusson, M., Sousa, R., & Ma, P. (2012). Secondary production as a tool for better understanding of aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences, 69*(7).

Douglas, A. E. (2014). Symbiosis as a general principle in eukaryotic evolution. *Cold Spring Harbor Perspectives in Biology, 6*(2), 1–14. https://doi.org/10.1101/cshperspect.a016113

Elton, C. S. (1927). Animal Ecology. The Macmillan Company.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters, 10*(12), 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Endow, S. A. (2003). Kinesin motors as molecular machines. *BioEssays, 25*(12), 1212–1219. https://doi.org/10.1002/bies.10358

England, J. L. (2013). Statistical physics of self-replication. *The Journal of Chemical Physics, 139*(12), 121923. https://doi.org/10.1063/1.4818538

Flynn, D. F. B., & Wolkovich, E. M. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist, 219*(4), 1353–1362. https://doi.org/10.1111/nph.15232

Ge, Y., Lou, Y., Xu, M., Wu, C., Meng, J., Shi, L., & Xia, F. (2021). Spatial distribution and influencing factors on the variation of bacterial communities in an urban river sediment *. *Environmental Pollution, 272*(272). https://doi.org/10.1016/j.envpol.2020.115984

Geider, R. J., Delucia, E. H., Falkowski, P. G., Finzi, A. C., Grime, J. P., Grace, J., Kana, T. M., La Roche, J., Long, S. P., Osborne, B. A., Platt, T., Prentice, I.C., Raven, J. A., Schlesinger, W. H., Smetacek, V., Stuart, V., Sathyendranath, S., Thomas, R. B., Vogelmann,...Woodward, F.I. (2001). Primary productivity of planet earth: Biological determinants and physical constraints in terrestrial and aquatic habitats. *Global Change Biology, 7*(8), 849–882. https://doi.org/10.1046/j.1365-2486.2001.00448.x

Gorokhova, E., Rivetti, C., Furuhagen, S., Edlund, A., Ek, K., & Breitholtz, M. (2015). Bacteria-mediated effects of antibiotics on *Daphnia* nutrition. *Environmental Science and Technology, 49*, 5779–5787. https://doi.org/10.1021/acs.est.5b00833

Gratani, L. (2014). Plant Phenotypic Plasticity in Response to Environmental Factors. *Advances in Botany,* 2014.

Gray, M. W. (2017). Lynn Margulis and the endosymbiont hypothesis: 50 years later. *Molecular Biology of the Cell, 28*(10), 1285–1287. https://doi.org/10.1091/mbc.E16-07-0509

Harrison, E., & Brockhurst, M. A. (2012). Plasmid-mediated horizontal gene transfer is a coevolutionary process. *Trends in Microbiology, 20*(6), 262–267. https://doi.org/10.1016/j.tim.2012.04.003

Harvey, J. A., Van Dam, N. M., & Gols, R. (2003). Interactions over four trophic levels: foodplant quality affects development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *Journal of Animal Ecology, 72*, 520–531.

Herrera, C. M., & Bazaga, P. (2013). Epigenetic correlates of plant phenotypic plasticity: DNA methylation differs between prickly and nonprickly leaves in heterophyllous *Ilex aquifolium* (Aquifoliaceae) trees. *The Linnean Society of London, 171*, 441–452.

Higgs, P. G., & Lehman, N. (2015). The RNA World: Molecular cooperation at the origins of life. *Nature Reviews Genetics, 16*(1), 7–17. https://doi.org/10.1038/nrg3841

Hillebrand, H. (2004). On the Generality of the Latitudinal Diversity Gradient. *American Naturalist, 163*(2), 192–211.

Huxley, J. (1943). Evolution, the modern synthesis. New York; London: Harper & brothers.

Irigoien, X., Flynn, K. J., & Harris, R. P. (2005). Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact? *Journal of Plankton Research, 27*(4), 313–321. https://doi.org/10.1093/plankt/fbi011

Jaspers, C., Fraune, S., Arnold, A. E., Miller, D. J., Bosch, T. C. G., & Voolstra, C. R. (2019). Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Zoology, 133*(February), 81–87. https://doi.org/10.1016/j.zool.2019.02.007

Jenkins, D. G., & Pierce, S. (2017). General allometric scaling of net primary production agrees with plant adaptive strategy theory and has tipping points. *Journal of Ecology, 105*(4), 1094–1104. https://doi.org/10.1111/1365-2745.12726

Jia, J., Gao, Y., Zhou, F., Shi, K., Johnes, P. J., Dungait, J. A. J., Ma, M., & Lu, Y. (2020). Identifying the main drivers of change of phytoplankton community structure and gross primary productivity in a river-lake system. *Journal of Hydrology, 583*(April). https://doi.org/10.1016/j.jhydrol.2020.124633

Kaluthota, S., Pearce, D. W., Evans, L. M., Letts, M. G., Whitham, T. G., & Rood, S. B. (2015). Higher photosynthetic capacity from higher latitude: Foliar characteristics and gas exchange of southern, central and northern populations of Populus angustifolia. *Tree Physiology, 35*(9), 936–948. https://doi.org/10.1093/treephys/tpv069

King, E. G., & Roff, D. A. (2010). Modeling the evolution of phenotypic plasticity in resource allocation in wing-dimorphic insects. *American Naturalist, 175*(6), 702–716. https://doi.org/10.1086/652434

Krissansen-Totton, J., Olson, S., & Catling, D. C. (2018). Disequilibrium biosignatures over Earth history and implications for detecting exoplanet life. *Science Advances, 4*(eaao5747).

Kwiatkowski, L., Aumont, O., Bopp, L., & Ciais, P. (2018). The Impact of Variable Phytoplankton Stoichiometry on Projections of Primary Production, Food Quality, and Carbon Uptake in the Global Ocean. *Global Biogeochemical Cycles, 32*(4), 516–528. https://doi.org/10.1002/2017GB005799

Levin, S. (1992). The Problem of Pattern and Scale in Ecology. *Ecology, 73*(6).

Liang, J., Crowther, T. W., Picard, N., Wiser, S., Zhou, M., Alberti, G., Schulze, E. D., McGuire, A. D., Bozzato, F., Pretzsch, H., De-Miguel, S., Paquette, A., Hérault, B., Scherer-Lorenzen, M., Barrett, C. B., Glick, H. B., Hengeveld, G. M., Nabuurs, G. J., Pfautsch, S., … Reich, P. B. (2016). Positive biodiversity-productivity relationship predominant in global forests. *Science, 354*(6309). https://doi.org/10.1126/science.aaf8957

Lindeman, R. L. (1942). Experimental Simulation of Winter Anaerobiosis in a Senescent Lake Author (s): Raymond L . Lindeman Reviewed work (s): Published by: Ecological Society of America Stable URL : http://www.jstor.org/stable/1930867. *Ecology, 23*(1), 1–13.

Lindeman, R. L. (1942). The trophic-dynamic aspect of ecology. *Ecology, 23(*1), 399–418. papers2://publication/uuid/D33E68F1-904E-4DB6-AE8D-DE9B984D4B40

Locey, K. J., & Lennon, J. T. (2016). Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America, 113*(21), 5970–5975. https://doi.org/10.1073/pnas.1521291113

Ludovick Achat, D., Pousse, N., Nicolas, M., Bredoire, F., & Augusto, L. (2016). Soil properties controlling inorganic phosphorus availability: general results from a national forest network and a global compilation of the literature. *Biogeochemistry, 127*, 255–272. https://doi.org/10.1007/s10533-015-0178-0

Margulis, L., & Sagan, D. (2000). What is Life?. University of California Press.

Marquet, P. A., Quiñones, R. A., Abades, S., Labra, F., Tognelli, M., Arim, M., & Rivadeneira, M. (2005). Scaling and power-laws in ecological systems. *Journal of Experimental Biology, 208*(9), 1749–1769. https://doi.org/10.1242/jeb.01588

Mekonnen, M. M., & Hoekstra, A. Y. (2017). Global Anthropogenic Phosphorus Loads to Freshwater and Associated Grey Water Footprints and Water Pollution Levels: A High-Resolution Global Study. *American Geophysical Union*, 345–358. https://doi.org/10.1002/2017WR020448

Metcalfe, D. B., Hermans, T. D. G., Ahlstrand, J., Becker, M., Berggren, M., Björk, R. G., Björkman, M. P., Blok, D., Chaudhary, N., Chisholm, C., Classen, A. T., Hasselquist, N. J., Jonsson, M., Kristensen, J. A., Kumordzi, B. B., Lee, H., Mayor, J. R., Prevéy, J., Pantazatou, K., … Abdi, A. M. (2018). Patchy field sampling biases understanding of climate change impacts across the Arctic. *Nature Ecology and Evolution, 2*(9), 1443–1448. https://doi.org/10.1038/s41559-018-0612-5

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., & Hairston, N. G. Jr. (2012). Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society B, 279,* 1873–1882. https://doi.org/10.1098/rspb.2011.2404

Ng'oma, E., Perinchery, A. M., & King, E. G. (2017). How to get the most bang for your buck: The evolution and physiology of nutrition-dependent resource allocation strategies. *BioRxiv*. https://doi.org/10.1101/113027

O'Malley, M. A. (2014). Philosophy of Microbiology. Cambridge University Press.

Peacock, K. A. (2011). The three faces of ecological fitness. Studies in History and Philosophy of Science Part C: *Studies in History and Philosophy of Biological and Biomedical Sciences, 42*(1), 99–105. https://doi.org/10.1016/j.shpsc.2010.11.011

Pigliucci, M. (2002). Are ecology and evolutionary biology "soft" sciences? *Annales Zoologici Fennici, 39*(2), 87-98. Retrieved June 19, 2021, from http://www.jstor.org/stable/23735814

Raia, P., Carotenuto, F., Mondanaro, A., Castiglione, S., Passaro, F., Saggese, F., Melchionna, M., Serio, C., Alessio, L., Silvestro, D., & Fortelius, M. (2016). Progress to extinction: Increased specialisation causes the demise of animal clades. *Scientific Reports, 6*(0316), 1–10. https://doi.org/10.1038/srep30965

Reed, D. C., Algar, C. K., Huber, J. A., & Dick, G. J. (2014). Gene-centric approach to integrating environmental genomics and biogeochemical models. *Proceedings of the National Academy of Sciences of the United States of America, 111*(5), 1879–1884. https://doi.org/10.1073/pnas.1313713111

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology, 14*(3), 255–274. http://www.ncbi.nlm.nih.gov/pubmed/11541390

Sanders, D., Moser, A., Newton, J., & Frank van Veen, F. J. (2016). Trophic assimilation efficiency markedly increases at higher trophic levels in four-level host–parasitoid food chain. *Proceedings of the Royal Society B: Biological Sciences, 283*(1826). https://doi.org/10.1098/rspb.2015.3043

Schramski, J. R., Dell, A. I., Grady, J. M., Silby, R. M., Brown, J. H., Sibly, R. M., & Brown, J. H. (2015). Metabolic theory predicts whole-ecosystem properties. *Proceedings of the National Academy of Sciences, 112*(8), 201423502. https://doi.org/10.1073/pnas.1423502112

Shurin, J. B., Gruner, D. S., & Hillebrand, H. (2006). All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proceedings of the Royal Society B: Biological Sciences, 273*(1582), 1–9. https://doi.org/10.1098/rspb.2005.3377

Sison-Mangus, M. P., Mushegian, A. A, & Ebert, D. (2015). Water fleas require microbiota for survival, growth and reproduction. *The ISME Journal, 9*(1), 59–67. https://doi.org/10.1038/ismej.2014.116

Smetacek, V. (2001). A watery arms race. Nature, 411, 2001.

Stewart, J. E. (2019). The Origins of Life: The Managed-Metabolism Hypothesis. *Foundations of Science, 24*(1), 171–195. https://doi.org/10.1007/s10699-018-9563-1

Suárez, J., & Triviño, V. (2019). A metaphysical approach to holobiont individuality: Holobionts as emergent individuals. *Quaderns de Filosofia, 6*(1), 59. https://doi.org/10.7203/qfia.6.1.14825

Taipale, S. J., Kankaala, P., Tiirola, M., & Jones, R. I. (2008). Whole-lake dissolved inorganic 13C additions reveal seasonal shifts in zooplankton diet. *Ecology, 89*(2), 463–474. https://doi.org/10.1890/07-0702.1

Tams, V., Helene, J., Anne, N., & Mathilde, E. (2020). Insights into the genetic basis of predator-induced response in *Daphnia galeata*. *Ecology and Evolution, 10*, 13095–13108. https://doi.org/10.1002/ece3.6899

Tilman, D., Reich, P. B., & Isbell, F. (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proceedings of the National Academy of Sciences of the United States of America, 109*(26), 10394–10397. https://doi.org/10.1073/pnas.1208240109

Toledo, M., Poorter, L., Peña-Claros, M., Alarcón, A., Balcázar, J., Leaño, C., Licona, J. C., Llanque, O., Vroomans, V., Zuidema, P., & Bongers, F. (2011). Climate is a stronger driver of tree and forest growth rates than soil and disturbance. *Journal of Ecology, 99*(1), 254–264. https://doi.org/10.1111/j.1365-2745.2010.01741.x

Vailati-Riboni, M., Palombo, V., & Loor, J. J. (2017). What Are Omics Sciences? In B. N. Ametaj (Ed.), Periparturient Diseases of Dairy Cows: A Systems Biology Approach (pp. 1–7). *Springer International Publishing*. https://doi.org/10.1007/978-3-319-43033-1_1

Vandegehuchte, M. B., Vandenbrouck, T., Coninck, D. D., Coen, D., & W.M., J. (2010). Can metal stress induce transferable changes in gene transcription in *Daphnia magna*? Aquatic Toxicology, 97, 188–195.

Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., Oikos, S., May, N., Violle, C., Navas, M., Vile, D., Kazakou, E., & Fortunel, C. (2007). Let the concept of trait be functional! *Oikos, 116*(5), 882–892.

Waddington, C. H. (1940). Organizers and Genes. Cambridge University Press.

Waddington, C. H. (1957). The Strategy of the Genes. London: Geo Allen & Unwin.

Weiss, L. C. (2019). Sensory Ecology of Predator-Induced Phenotypic Plasticity. *Frontiers in Behavioral Neuroscience, 12(January)*, 1–12. https://doi.org/10.3389/fnbeh.2018.00330

Weiss, L., Laforsch, C., & Tollrian, R. (2012). The taste of predation and the defences of prey. *In Chemical Ecology in Aquatic Systems* (Issue April 2015). https://doi.org/10.1093/acprof

Woese, C. R., & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America, 74*(11), 5088–5090.

Woese, C. R., Kandlert, O., & Wheelis, M. L. (1990). Towards a natural system of organisms: Proposal for the domains. *Proceedings of the National Academy of Sciences of the United States of America, 87*(June), 4576–4579.

Woese, C. R. (2004). A new biology for the 21st century. *Microbiology and Molecular Biology Reviews*, 173–186. https://doi.org/10.1890/1540-9295-7.9.455

Wollenberger, L., Halling-Sørensen, B., & Kusk, K. O. (2000). Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. *Chemosphere, 40*(7), 723–730. https://doi.org/10.1016/S0045-6535(99)00443-9

Xue, R., Ma, Q., Baker, M. A. B., & Bai, F. (2015). A Delicate Nanoscale Motor Made by Nature—The Bacterial Flagellar Motor. *Advanced Science, 2*(9), 1–7. https://doi.org/10.1002/advs.201500129

Zhang, Y., Ma, K., Anand, M., & Fu, B. (2006). Do generalized scaling laws exist for species abundance distribution in mountains? *Oikos, 115*(1), 81–88. https://doi.org/10.1111/j.2006.0030-1299.15021.x

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews, 32*(5), 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

Zvereva, E. L., & Kozlov, M. V. (2021). Biases in ecological research: attitudes of scientists and ways of control. *Scientific Reports, 11*(1), 1–9. https://doi.org/10.1038/s41598-020-80677-4

Chapter 2 : Environmental and Ecological Disturbance as Drivers of Antibiotic Resistant Bacterial Communities Across an Urban Riverscape

Angela M. Klock[1], Marilyn C. Roberts[2], Christian E. Torgersen[3], Daniel J. Vogt[1], Kristiina A. Vogt[1]

[1] School of Environmental and Forest Sciences, University of Washington, Seattle WA, U.S.A. 98195

[2] Department of Environmental & Occupational Health Sciences, University of Washington, Seattle WA, U.S.A. 98195

[3] U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Cascadia Field Station, University of Washington, Seattle WA, U.S.A. 98195

Freshwater Biology (In revision)

Abstract

Antibiotic resistant bacteria are prevalent environmental contaminants in freshwaters. The increase of antibiotic resistant pathogens threatens public health through direct and indirect exposure because these organisms are ubiquitous in urban surface waters, and antibiotic resistance genes circulate throughout the urban water cycle. Natural resource managers need information on spatial patterns of antibiotic resistant bacteria and associated ecological factors to improve water quality monitoring and assess public health risks. To gain insights into how antibiotic resistant bacteria are distributed across an urban riverscape, we collected water and epilithic biofilm samples and measured four physicochemical parameters at 29 sites in the Green-Duwamish River basin, Washington, USA. We investigated catchment-wide longitudinal patterns of fecal indicator bacteria (Gram-negative coliforms and *Escherichia coli*) and hypothesized that the presence of antibiotic resistance would be associated with environmental heterogeneity, bacterial traits, and stream type. Sampling sites were distributed from the mouth of the river to 137 km upstream. Antibiotic resistance was determined by microbial growth on MacConkey agar supplemented with three different antibiotics (ampicillin, chloramphenicol, or tetracycline). MacConkey agar selectively isolates enteric bacteria and differentiates *E. coli* from other coliforms based on lactose fermentation. The concentration of *E. coli* in water samples was positively associated with antibiotic resistance of coliforms in the upper sub-catchment ($p < 0.001$), and positively associated with antibiotic resistance of *E. coli* ($p = 0.015$) and coliforms ($p < 0.001$) in the lower sub-catchment. Phenotypic antibiotic resistance was positively associated with urban stressors in downstream sites, but antibiotic resistance was also detected in pristine river segments (82% overall detection rate, i.e., 24 out of 29 sites [17 in the main stem and 7 in tributaries]). Phenotypic resistance was significantly higher ($p < 0.05$) among coliform bacteria in the water column of

tributaries draining moderate to extensively developed sub-catchments. Generalized linear mixed-effects models showed that 22% of the variance in the presence of antibiotic resistance was explained by fixed effects (environmental variability, stream type, lactose metabolism) and 39% was explained by both fixed effects and differences between the sub-catchments. Our study provides new evidence that environmental heterogeneity, bacterial metabolic traits, and stream type are important underlying factors associated with the spatial patterns of antibiotic resistant coliform bacteria. The holistic riverscape approach that we employed made it possible to quantify the distribution of antibiotic resistance within an urbanized basin with extensive non-point sources of anthropogenic pollution.

Key words: antibiotic resistance, Gram-negative, bacterial ecology, riverine ecology, urban

1. Introduction

The "urban river syndrome" describes the vulnerability of rivers to the physical, chemical, and biological impacts of urban development (Walsh et al., 2005). Urbanization has fundamentally changed the way water moves across the landscape and has resulted in extensive contamination of urban rivers. Urban rivers receive continuous inputs of non-point source pollutants including sediments, toxics, nutrients, and fecal coliform bacteria from the waste of humans, domestic animals and wildlife. Coliform bacteria are found in the intestinal tract of humans and other warm-blooded animals and live in aquatic environments and soils (Rompré et al., 2002), and commonly function both as beneficial human commensals and pathogens (Tadesse et al., 2012).

Urban rivers contaminated with fecal bacteria pose a significant public health threat through direct (swimming or wading) and indirect exposure (fishing, pets, clothing) to pathogens. Many fecal coliforms persist in freshwater habitats as transient constituents entrained in the water column or as members of epilithic biofilm communities. Typically, municipal water quality assessments are made by quantifying coliform bacteria, but this provides little information on how pollutants impact microbial community structure and in-stream ecological function (Bernhardt & Palmer, 2007). The mixing of anthropogenic fecal bacteria and pathogens with environmental bacteria increases the likelihood that novel and clinically relevant antibiotic resistance (AR) mechanisms will develop in human commensals and pathogens even in the absence of environmental selective pressure (Karkman et al., 2019). These new antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) can then spread from anthropogenic (exogenous) or environmental (endogenous) niches to surface and ground water, thus entering the urban water cycle. Therefore, it is crucial to address the prevelance and distribution of ARB and ARGs in receiving rivers (Rousham et al., 2018).

The "urban river resistome" is a reservoir of resistance genes that has arisen due to the mixing of naturally occurring resistance traits and bacteria originating from human and animal waste along with the selective pressure of pollutants which can co-select for multiple resistant genes (Wellington et al., 2013). Bacterial AR is increasingly reported to occur in aquatic systems worldwide (MacFadden et al., 2018; Martínez, 2012). Although ARB and ARGs are part of nature and generally detected at low levels in natural environments (Davies, 2006; Singer et al., 2006), their spatial patterns are often unpredictable. For example, ARB have been isolated from 'pristine habitats' with no legacy of antibiotic contamination (De Souza et al., 2006; Eisenberg et al., 2012; Garcia-Armisen et al., 2011). Whereas ARGs occur naturally, urbanization (Zhou et al., 2017) and agriculture (Thanner et al., 2016) increase the diversity and prevalence of ARGs in receiving rivers, and these traits alter the ecological structure and function of microbial communities (Keen & Patrick, 2013; Rosi et al., 2018a). There are significant knowledge gaps in the basic ecology of bacterial antibiotic resistance in urban rivers, such as which metric to use to evaluate resistance and at what scale of observation; longitudinally (i.e., main stem versus tributary), which stream compartment (water, biofilm) and which bacterial functional groups (heterotrophic, commensal, pathogenic).

In response to the global health emergency of increasing AR, two important global frameworks for expanding interdisciplinary collaborations have been developed. The One Health Initiative (www.onehealthinitiative.com) and the EcoHealth Alliance (www.ecohealthalliance.org) propose that human behavior and epidemiological dynamics determine human, animal, and environmental health, and therefore should be studied in their interconnected and interdependent contexts. For example, One Health emphasizes biomedical research, education, and clinical care of zoonotic diseases, whereas EcoHealth focuses on disease prediction and prevention through conservation (Roger et al., 2016). In response to the expected increase in deaths from AR infections,

the World Health Organization launched a global action plan on Antimicrobial Resistance in 2015 which placed emphasis on developing risk assessments to address environmental contamination by determinants that increase bacterial antibiotic resistance (www.who.int/antimicrobial-resistance/global-action-plan/en) (World Health Organization, 2014). Although ecologically centered approaches are increasingly applied in clinical and public health settings, the role of the urban river water cycle in the fate of AR traits across multiple ecological domains remains unclear (Sanderson et al., 2018). Urban rivers are socio-ecological 'commons' that are subject to multiple stressors. These rivers provide critical cultural and psychological ties to the natural world but are also extensively contaminated and provide the conditions that amplify antibiotic resistance. In spite of their critical importance and the urgency of the antibiotic resistance crisis for human health, there is limited regulatory monitoring of ABR or ARGs in urban rivers. Furthermore, few studies have applied ecological principles (McGill, 2019) and natural selection (Martinez, 2014) to map the urban river resistome. The application of these concepts to identify the catchment-wide spatial patterns of ARB will improve assessment of the health risks associated with urban rivers, (Ouyang et al., 2015; Watkinson et al., 2007).

Antibiotic resistant bacteria are commonly detected throughout entire river networks and in low-order tributaries (Amos et al., 2015; Winkworth, 2013; Zhang et al., 2009). This may be due to the vulnerability of tributaries to anthropogenic impacts based on their proximity to pollution sources, such as on-site septic systems, and the poor assimilation capacity of smaller streams (Yang et al., 2017). Most municipal regulatory sampling protocols are conducted within the main stem or large tributaries; therefore, smaller tributaries have not been sufficiently monitored for coliform bacteria. Because low-order streams provide substantial linkages between terrestrial and aquatic environments and are more sensitive to changes in land use, they provide unique habitats for communities of prokaryotes and eukaryotes and have a strong influence on the structure of bacterial

communities (Findlay et al., 2008). Bacterial communities found in tributaries are also strongly influenced by hydrology (Katharina Besemer et al., 2009), watershed characteristics (Brendel & Soupir, 2017), and the degree of urbanization (Hosen et al., 2017). Furthermore, urban development near river headwaters is correlated with higher nutrients and coliform counts, which tend to decrease secondary production downstream (Freeman et al., 2007). This suggests that the amount of coliform bacteria in the main stem of a river may scale with stream network density (i.e., the number of tributary confluences) (Ferguson et al., 2006; Wu, 2004) and level of urbanization.

Different stream compartments host unique bacterial communities. Bacteria associated with the water column are more diverse as they arise from soils that constitute multiple continuous inputs to the river (Crump et al., 2012), but these bacteria are largely biologically inactive (Edwards et al., 1990). Conversely, bacterial communities in biofilms are generally less diverse but are sites of biological productivity with stable bacterial compositions (Besemer et al., 2012, Flemming et al., 2016). Due to the unique structure and function of these modes of life, water-associated bacteria and epilithic biofilms represent distinct ecological niches that exhibit different physiological responses to the selective pressures imposed by urbanization (Fletcher, 1992; Leff et al., 1992). Therefore, it is likely that environmental physicochemical gradients will result in unique spatial patterns among ARB dependent upon the stream compartment with which they are associated.

Many Gram-negative coliforms are well-known human and animal pathogens that are treated with a broad-spectrum of classes of antibiotics. Members of the bacterial family Enterobacteriaceae can be distinguished by assessing their genetic relatedness or similarity through their essential metabolic functions, e.g., carbohydrate metabolism, which are usually encoded on the genome (Kreimer et al., 2008). Gram-negative organisms that can ferment the sugar lactose include *Escherichia coli (E. coli*), which is a common indicator species of anthropogenic fecal contamination, and which is also known to carry multiple resistance genes (Pontes et al., 2009; Yang et al., 2004).

Gram-negative bacteria that do not ferment lactose include many bacterial genera commonly found in freshwater such as *Acinetobacter, Aeromonas, Enterobacter* and *Pseudomonas* (Ash et al., 2002).

Many urban rivers receive relatively constant high concentrations of pollutants from point-sources, such as commercial and industrial businesses, agricultural operations, municipal wastewater treatment plants, landfills, and transportation maintenance facilities. Most municipal surface water monitoring occurs near these facilities. Conversely, non-point sources of pollution are difficult to quantify because they are distributed diffusely over a large area. Most of these contaminants are delivered intermittently after precipitation events. However, few studies have been conducted to identify the influence of non-point source pollution in urban rivers (Rosi-Marshall et al., 2015). A critically important emergent property of rivers is the ability to be self-cleansing. This process is driven by natural river morphometry, environmental heterotrophic bacteria, and high dissolved oxygen. When rivers are urbanized, water temperature and sediments increase, flow decreases, and dissolved oxygen and substrate rugosity decline, resulting in a loss of self-cleansing capacity. These changes in environmental conditions may cause shifts in microbial community structure and amplify antibiotic resistance (Almakki et al., 2019). By quantifying the relationships between environmental conditions and ARB and ARG occurrence, it may be possible to identify potential 'hotspots' for antibiotic resistance along rivers and prioritize monitoring and restoration efforts (Allen et al., 2010; Prause, 2014).

The aim of this study was to investigate the relationship between riverine environmental conditions and spatial patterns of phenotypic resistance. We utilized standard water quality testing methods to focus on three classes of antibiotics (ampicillin, chloramphenicol, and tetracycline) among commonly monitored indicator bacteria in an urban riverscape that has no major point-sources of anthropogenic pollution. We hypothesized that (1) the prevalence ARB would increase with urbanization and in tributaries; (2) the spatial patterns of ARB would reflect distinct responses

to environmental conditions based on bacterial ecology (i.e., lactose metabolism [*E. coli*, lactose positive versus coliforms, lactose negative]) or mode of life (water-associated versus biofilm); (3) anthropogenic water-borne *E. coli* would dominate the microbial community in proximity to urbanized areas and agriculture with downstream accumulation; and (4) non-*E. coli* coliforms would be heterogeneously distributed across the entire basin due to their ability to exploit a wider range of niches.

2. Methods

2.1. Study area

The Green-Duwamish River is a fourth-order basin located in western Washington State, USA (Fig. 2-1). The catchment drains 1,274 km^2 with a diverse gradient of land uses from pristine to highly urbanized and has a population of 400,000 (Easthouse, 2008). The King County Department of Natural Resources delineated this basin into upper, middle, and lower sub-catchments (https://www.govlink.org/s/9/activities-partners/default.aspx#map). The upper sub-catchment has headwaters in a protected forest and flows 40 km west to the Howard Hansen Reservoir. This part of the basin provides the municipal water supply for the city of Tacoma. The middle sub-catchment is a mosaic of cultivated pastures, grasslands, commercial forestry operations, and residential and commercial development, with approximately thirty dairy farms (> 300 cows per farm). The lower sub-catchment is largely an industrial development zone where the Green River changes in name to the Duwamish, which is the Native American name for this section of the river. The lower Duwamish River is an important economic and regional transportation corridor that is extensively channelized and has been heavily polluted by industrial development over the past 100 years (Shaw & Liu, 2005). The lowermost 8.7 km of the Duwamish River was designated as a U.S.

Environmental Protection Agency Superfund site in 2001, but this section is still a migration corridor for eight species of Pacific salmon and trout (King County Department of Natural Resources, 2000).

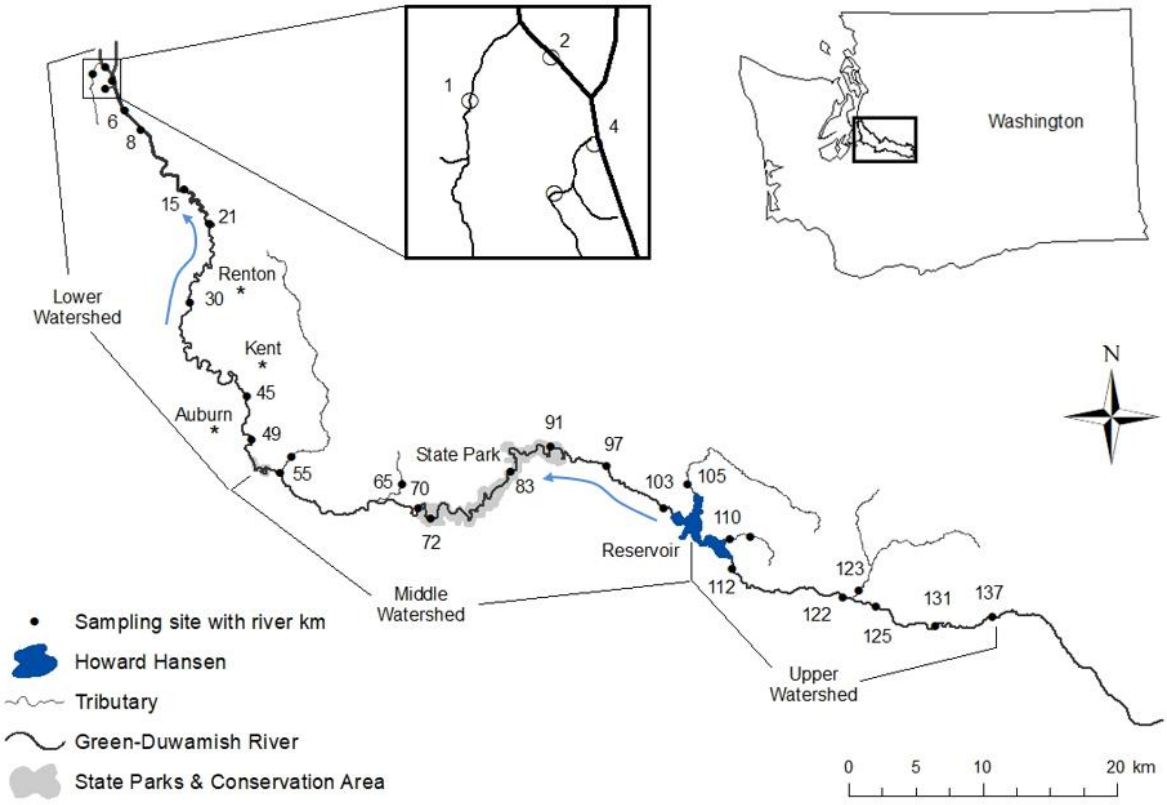


Figure 2-1: Green-Duwamish River in Washington (USA) with the upper, middle, and lower sub-catchments demarcated by brackets. State parks and the cities of Renton, Kent, and Auburn are delineated. Sampling sites on the main stem and tributaries of the Green-Duwamish River are annotated with upstream distance from the mouth of the river in kilometers.

There are no wastewater treatment plant effluents or other major point-source pollution on the Green-Duwamish River. However, a 2014 Washington Department of Ecology assessment of water quality reported that multiple segments of the middle and lower sub-catchments of the river did not meet water quality standards for temperature and fecal coliform bacterial counts (Department of Ecology, 2014).

2.2. Data collection and processing of water and biofilm samples

Water and epilithic biofilm samples were collected in duplicate at 29 sites (22 main stem and 7 tributary) from the mouth of the river to 137 km upstream. Samples were collected during base-flow (stable) conditions 15-Aug-2014 to 15-Sept-2014. Water samples were collected in 120 ml sterile bottles at 20 cm depths near the riverbed using the water grab method. Epilithic biofilms were collected by abrasion over the upper surface of the stream substratum with a sterile Whirl-Pak®Speci-Sponge® that was inserted into a Whirl-Pak®Sampling Bag (Nasco, Fort Atkinson, WI, USA). Samples were stored on ice and processed within 12 h.

Water temperature and specific conductance (conductivity standardized to 25 °C) were measured at each site with a multi-parameter YSI 85 (YSI Inc., Yellow Springs, Ohio). Stream power (shearing force on the substrata) and channel sinuosity (ratio of stream length to valley length) were calculated in a geographic information system (GIS) from a 10-m digital elevation model (DEM) of King County and high-resolution satellite and aerial imagery from Digital Globe (http://goto.arcgisonline.com/maps/World_Imagery). These metrics were calculated for each sample site, and the site data were then calculated for the upper, middle, and lower sub-catchments. All geospatial processing was conducted with ArcGIS 10.1 (ESRI, 2014).

In the laboratory, water samples were not filtered before processing. Biofilms from each Whirl-Pak®Speci-Sponge® Sampling Bag were processed by adding 25 ml of sterile deionized (DI)

water to the Whirl-Pak and manually massaging the bag for 2 min to release the bacterial matrix from the sponge into the liquid. The liquid was then transferred into a sterile 50 ml Falcon tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA). Ten sterile 2 mm glass beads (Carolina Biological, Burlington, North Carolina, USA) were added to the tube and the tube was vortexed for three min to break up the biofilm. The sample was then filtered through sterile 0.45 μm filter paper (Thomas Scientific, Swedesboro, NJ, USA) into a sterile glass bottle. Additional DI water was added to each bottle to obtain a final volume of 100 ml.

2.3. Enumeration of coliforms and *E. coli* from water and biofilms

Viable total coliform and *E. coli* counts were assayed using the 24-hour Colilert method, with Quanti-Tray/2000 according to the manufacturer's instructions. This assay determines the most probable number (MPN) of total coliform and *E. coli* (IDEXX Laboratories, Inc., Westbrook, Maine, USA). A total of fifty-eight 96-well trays, i.e., 29 water and 29 biofilms, were prepared. The IDEXX wells were assessed following the method outlined by IDEXX: (1) clear IDEXX wells were marked as negative for any coliforms, (2) yellow-colored wells contained coliforms, and (3) yellow-colored wells that fluoresced under ultraviolet light contained a mixture of coliforms and *E. coli* (https://www.idexx.com/en/water/). The MPN results were calculated with the IDEXX MPN Generator 3.2 software provided by the manufacturer.

2.4. Screening of antibiotic resistant bacteria

Screening for antibiotic resistance was accomplished by withdrawing 1.0 ml aliquots from each IDEXX Quanti-tray. From each tray, two wells that were yellow and fluoresced (i.e., coliforms + *E. coli*) were selected; if there were not two fluorescent wells, a yellow well (i.e., coliform only) was selected in its place. Seven sites in the most polluted river segments required three 10-fold serial

dilutions to obtain counts within the range of the IDEXX system. The back of the Quanti-tray was sprayed with 70% ethanol and incised with a sterile scalpel, and a 100 μl aliquot was withdrawn and placed into a sterile 2 ml Eppendorf tube. A 10-fold dilution was made using sterile DI water and 10 μl aliquots were spread on each of four MacConkey agar plates in duplicate: a control, and one plate each supplemented with either 25 μg/ml of ampicillin (Amp), chloramphenicol (Cm), or tetracycline (Tet).

The three antibiotics have different modes of action: (1) ampicillin inhibits cell wall production and is the most widely prescribed class of antibiotic; (2) tetracycline inhibits protein synthesis by binding the 30S ribosome subunit and is widely used in human and veterinary medicine but is becoming less desirable due to growing resistance; and (3) chloramphenicol inhibits protein synthesis by binding to the 50S subunit of the ribosome and has been widely prescribed but is no longer used in the U.S. due to safety concerns. Florfenicol is an antibiotic closely related to chloramphenicol that continues to be widely used in the beef and dairy industries. *Escherichia coli* is commonly resistant to multiple antibiotics, and simultaneous resistance to ampicillin, tetracycline, chloramphenicol and florfenicol is frequently reported (Nazaret & Aminov, 2014). Therefore, runoff from animal agriculture could perpetuate resistance to all these antibiotics in urban rivers. Due to the lack of data on the prevalence of antibiotic residues in the current river system, an intermediate breakpoint was selected for each antibiotic (25 ug/ml) in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2015).

All MacConkey plates were incubated at 36 °C for 24 h and then examined for growth. If incubated plates contained cells too numerous to count, a small number of cells were gathered with a sterile loop, mixed in a sterile 2 ml tube with 1.5 ml sterile water, and re-plated onto MacConkey plates. Those plates were then incubated for 24 h and examined for growth. After incubation, a subset of lactose positive colonies (collected as *E. coli*) and lactose negative colonies (collected as

coliforms) were haphazardly selected and streaked onto fresh plates. All individual colonies were tested against the three antibiotics at the given concentrations. When cultured on MacConkey agar, lactose fermenters (lactose positive bacteria) formed pink-red colonies, whereas non-lactose fermenters (lactose negative bacteria) formed clear colonies; the different colors facilitate differentiation among the metabolic groups.

2.5. Statistical analysis

Pairwise Pearson's product-moment correlation coefficients were calculated to evaluate multicollinearity between the environmental variables (temperature, specific conductance, stream power, channel sinuosity) and to test the strength of the environmental gradient to river distance at the catchment and sub-catchment scale. A series of Welch two-sample t-tests were used to compare each variable for differences between the main stem and tributaries stratified by sub-catchment. Coefficient of variation (CV) $[CV = sd/\bar{x} * 100]$ was calculated for each environmental variable by sub-catchment and stream type, where sd is the standard deviation and $\bar{x}$ is the sample mean. The CV for all four variables was summed as a measure of total environmental variability.

To investigate how microbial ecology influenced the distribution of ARB, we evaluated two factors. The first factor was mode of life or the "stream compartment" from which the bacterial cells were collected. The second factor was the ability to ferment lactose as a measure of metabolic diversity (phylogenetic divergence) and primary ecology, i.e., anthropogenic versus environmental origins of the bacteria (Fani, 2012). The presence of ARB was scored based on a binomial outcome (1 if present, 0 if absent). To determine if the level of antibiotic resistance varied by environmental and ecological factors, an antibiotic resistance profile was calculated for each sample with the following formula: a/b, where a is the number of antibiotics to which the sample was resistant and b is the number of antibiotics against which the sample was tested (Krumperman, 1983). Spatial

patterns of ARB were evaluated at two spatial scales: stream type (i.e., main stem versus tributary), and sub-catchment (lower, middle, or upper).

The detection of antibiotic resistant phenotypes was represented as the probability of occurrence with local polynomial regression fitting (LOESS) and 95% confidence intervals using the function `'geom_smooth'` from the R-package `'ggplot2'` (Wickham, 2016). The proportion of antibiotic resistant samples to total samples tested was evaluated with a three-sample test for equality of variance with a post hoc pair-wise comparisons with Levene's test to determine the probability of detecting resistance for each factor (29 sites, stream compartment, and lactose metabolism). The proportion of samples resistant to each individual antibiotic was calculated with a 3-sample test for equality of proportions with a post hoc analysis of variance (ANOVA) and Tukey multiple comparisons to detect differences between means at the 95% family-wise confidence level. A series of Spearman's rank correlation coefficients (r) was calculated to measure the association between the antibiotic resistance profile and the MPN of *E. coli* (exogenous contamination) in each sub-catchment. Wilcoxon rank sum tests were used to evaluate differences among factors.

To evaluate the association between environmental variables and ARB, two generalized linear models were fitted to the antibiotic resistance response data. A mixed-effect modeling approach was used because it relaxes an assumption of homoscedasticity and provides an independent evaluation of the mean and variance by partitioning the variance between fixed and random explanatory variables. The models were used to quantify the variance attributable to the fixed and random effects for each response at two spatial scales. A model assumption was that environmental conditions influenced the distribution of ARB, and therefore their spatial patterns would be nonrandom at one or more spatial scales. Summed environmental CV values were compared with asymptotic test for equality of coefficients of variation (Feltz & Miller, 1996).

All statistical analyses were conducted in the programming language R version 1.0.136 (R Core Team, 2020). Generalized linear mixed models (GLMMs) were fitted to the response data using the functions `'glmer'` and `'glmer.nb'` included in the R-package `'lme4'` (Bates et al., 2015). Each model included the CV and categorical variables as the fixed effects. Categorical factors consisted of stream compartment (binomial variable where water = 1 and biofilm = 2), lactose metabolism (binomial variable where coliforms [unable to ferment lactose] =1, and *E. coli* [able to ferment lactose] = 2), and stream type (binomial variable where main stem = 1 and tributary = 2). All models used sampling site within sub-catchment as a random spatial effect. Two GLMMs using a binomial error structure and logit link were fit to the antibiotic resistance response. If resistance to any of the three antibiotics was detected, it was reported as presence/absence data. The antibiotic resistance profile was modeled as the proportion of samples that were antibiotic resistant to the number of samples tested. Baseline models were constructed by comparing the main effects and the spatial random effect. Models were fit with backward elimination and forward selection of all terms and interactions, and 95% confidence intervals were calculated with non-parametric bootstrapping for mixed models. Model performance was evaluated by assessing the convergence of all parameter estimates, comparing the variability of the parameter estimates, and by examining the range of the confidence intervals. We use the Akaike information criteria (AIC) to identify the best approximating model (Burnham & Anderson, 2002).

3. Results

Urbanization was positively associated with temperature and environmental variability at the basin-scale, but the spatial patterns of ARB were non-linear at the sub-catchment scale and varied significantly by stream type, stream compartment, and lactose metabolism. Stream power and channel sinuosity varied throughout the surveyed river segments, with conductivity significantly

higher in the main stem near the mouth of the river due to mixing with estuarine waters and temperature was higher in the main stem and increasing downstream in both stream types (Table 2-1). Temperature was correlated with river distance (Pearson correlation, $r = 0.82$, $p < 0.000$), and main stem and tributary temperatures were significantly different in the lower sub-catchment ($t = 2.19$, $p = 0.035$) and the middle sub-catchment ($t = 4.65$, $p < 0.000$). Specific conductance was significantly higher in the lower sub-catchment compared to the other main stem sub-catchments ($t = 3.75$, $p = 0.001$). Stream power and channel sinuosity were highest in the middle sub-catchment. The CV (indicative of environmental variability) increased downstream and was lower in tributaries compared to the main stem in all sub-catchments. The lowest CV values were in upper sub-catchment tributaries (Table 1). There were significant differences in CV among sub-catchments ($S = 149.38$, $p < 0.001$) and between stream types ($S = 12.38$, $p < 0.001$).

Table 2-1: Bootstrapped sample mean, standard error and 95% confidence intervals of four environmental variables, and the summed coefficient of variation (CV) for each variable in the main stem and tributaries by sub-catchment.

	Lower		Middle		Upper	
	Main stem ($\bar{x}$ ± SE CI)	Tributary ($\bar{x}$ ± SE CI)	Main stem ($\bar{x}$ ± SE CI)	Tributary ($\bar{x}$ ± SE CI)	Main stem ($\bar{x}$ ± SE CI)	Tributary ($\bar{x}$ ± SE CI)
Temperature (˚C)	17.0 ± 0.17 16.7-17.4	16.6 ± 0.41 15.8-17.2	16.2 ± 0.21 15.9-16.7	15.27 ± 0.46 14.1-16.0	12.28 ± 0.29 11.7-12.8	12.5 ± 0.12 12.2-12.6
Conductivity (µS/cm-1)	150.7 ± 32.8 100.3-235.8	20.65 ± 7.40 5.8-28.1	78.56 ± 10.25 60.4-102.8	61.4 ± 4.68 52.0-68.5	48.6 ± 2.44 43.4-53.0	52.7 ± 0.05 52.6-52.8
Stream power (log10)	9.10 ± 0.17 8.8-9.5	9.55 ± 0.37 9.0-10.4	10.95 ± 0.39 10.0-11.6	11.26 ± 0.40 10.5-12.1	10.4 ± 0.18 10.0-10.7	10.7 ± 0.08 10.6-10.8
Channel sinuosity	1.25 ± 0.06 1.2-1.4	1.14 ± 0.08 1.0-1.3	1.74 ± 0.14 1.5-2.0	1.27 ± 0.09 1.1-1.4	1.2 ± 0.04 1.1-1.3	1.26 ± 0.01 1.2-1.3
∑CV	211.5	205.8	161.4	136.6	97.2	21.1

The MPN of *E. coli* indicated moderate correlation with endogenous ARB in pristine environments (Spearman $r = 0.37$, $p < 0.001$) and heavily polluted segments ($r = 0.47$, $p < 0.001$), and weak correlated with exogenous ARB in heavily polluted segments ($r = 0.21$, $p < 0.02$). This metric was uninformative in moderately impacted segments for both exogenous and endogenous bacteria.

3.1. Most probable number of coliforms in the catchment

Coliform bacteria were detected in all sampling locations except for two sites located on the main stem (km 90.6 and 45.4) and one site located on a tributary (km 65.0) (Figure 2-1, Table A1). The MPN of total coliforms was greatest in the upper sub-catchment (geometric mean (GM) = 1.13 x 10^3 MPN/100 ml ± 3.57) and lowest in the middle sub-catchment (GM = 3.68 x 10^2 MPN/100 ml ± 5.08). The range of *E. coli* MPN was 2.2 x 10^1 MPN/100 ml ± 1.01 to 1.77 x 10^2 MPN/100 ml ± 20.5.

The MPN of *E. coli* was an order of magnitude higher (10^2-10^3) in tributaries over the main stem in the lower ($p = 0.03$) and upper ($p = 0.01$) sub-catchments but similar between stream types in the middle sub-catchment (see Table A1). There were strong correlations between *E. coli* MPN and the antibiotic resistance profile of endogenous bacteria in the lower and upper sub-catchments (Table 2-2).

Table 2-2: Spearman rank correlation coefficients for the association between *E. coli* most probable number (MPN) and the antibiotic resistance profile for exogenous and endogenous bacteria by sub-catchment. The alternative hypothesis is that true rho is not equal to zero.

Sub-catchment	Source	Spearman rank correlation coefficient ρ̇ (rho)	S[†]	*p*-value
Lower	Exogenous	0.210	302660	$p = 0.015$
	Endogenous	0.469	203550	$p < 0.001$
Middle	Exogenous	-0.021	294050	$p = 0.819$
	Endogenous	0.095	260640	$p = 0.302$
Upper	Exogenous	-0.122	165380	$p = 0.238$
	Endogenous	0.366	93491	$p < 0.001$

[†] S-statistic is reported from the cor.test() function: $(n^3 - n)\frac{1 - r}{6}$

Where *r* is Pearson's correlation coefficient on the ranked variables and *n* is equal to the sample size of variables.

3.2. Proportion of antibiotic resistant bacteria

Resistant bacteria were detected at 83% (24/29) of sites, with *E. coli* isolates resistant to one or more antibiotics at 48% (14/29) of sites, and coliform isolates resistant to one or more antibiotics at 69% (20/29) of sites. Duplicate plating for antibiotic resistance demonstrated 95% congruence between plate results. The probability of ARB presence increased in a downstream direction, and this was most notable for water-associated coliforms (Fig. 2-2A) and *E. coli* in biofilms (Fig. 2-2B).

There were significant differences in the proportion of AR coliforms in sub-catchments with differences between the lower-middle sub-catchments (Levene's test for homogeneity of variance, $p = 0.027$) and the lower-upper sub-catchments ($p < 0.001$). The proportion of AR coliforms in the water column was significantly higher ($p = 0.027$) in the lower sub-catchments, but overall ARB in biofilms were heterogeneously distributed across the catchment and did not differ significantly between coliforms and *E. coli* (Table 2-3).

Table 2-3: Proportion of antibiotic resistant bacterial communities detected in lower, middle, and upper sub-catchments. Results are for all exogenous and endogenous bacteria with antibiotic resistance detected (top row), in water (middle row), and in biofilms (lower row).

	Lower	Middle	Upper	Proportion test
All	27.3	15.0	8.3	X^2 (df = 2, N = 348) = 14.61, $p < 0.001$
Coliforms	18.2	8.3	5.2	X^2 (df = 2, N = 348) = 10.92, $p = 0.004$
E. coli	9.1	6.7	3.1	X^2 (df = 2, N = 348) = 3.25, $p = 0.203$
Water	34.8	11.7	4.2	X^2 (df = 2, N = 174) = 20.19, $p < 0.001$
Coliforms	24.2	6.7	4.2	X^2 (df = 2, N = 174) = 13.10, $p = 0.001$
E. coli	10.6	5.0	0.0	X^2 (df = 2, N = 174) = 5.86, $p = 0.053$
Biofilm	19.7	18.3	12.6	X^2 (df = 2, N = 174) = 1.08, $p = 0.581$
Coliforms	12.1	10.0	6.3	X^2 (df = 2, N = 174) = 1.08, $p = 0.579$
E. coli	7.6	8.3	6.3	X^2 (df = 2, N = 174) = 0.16, $p = 0.918$

The spatial patterns of ARB indicated a positive association with urbanization at the basin scale, but the prevalence of AR coliform bacteria was significantly different among sub-catchments in the water column. In contrast, AR *E. coli* were heterogeneously distributed across the catchment is both stream compartments (Table 2-3). Although ARB in biofilms generally increased in the downstream direction, there was no significant difference in their prevalence between *E. coli* and coliforms nor between sub-catchments (Table 2-3).

Bacterial isolates resistant to two or more antibiotics were observed at 41% of the sites (i.e., seven sites in the lower sub-catchment and five sites in the middle sub-catchment).

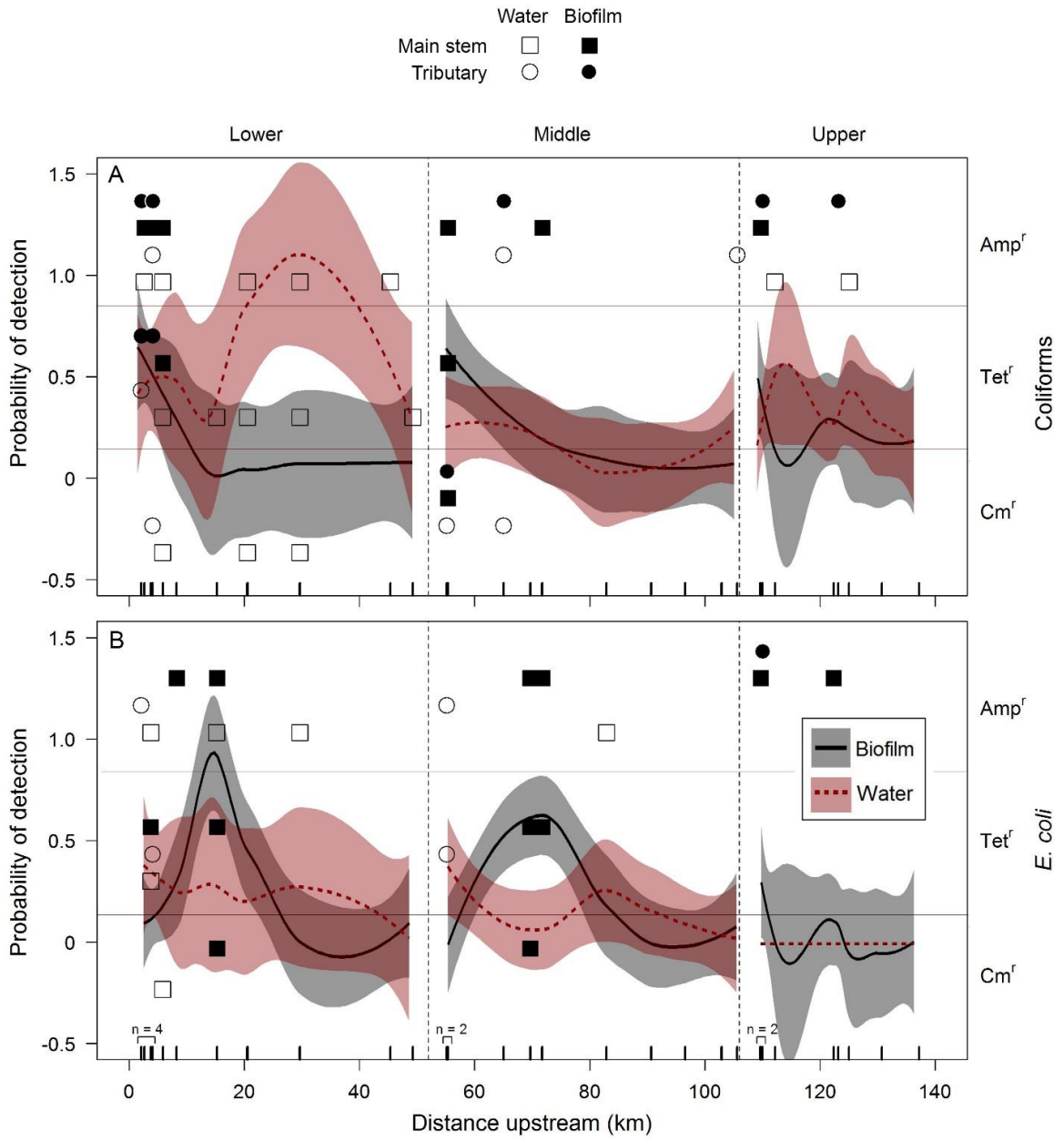


Figure 2-2: Spatial patterns of antibiotic resistance detected in water and biofilm samples (circles and squares) and predicted probability of detection (LOESS smoothing lines with 95% confidence intervals) of (A) coliforms and (B) *E. coli* resistant to ampicillin (Amp^r), chloramphenicol (Cm^r), and tetracycline (Tet^r) at 25ug/ml. Detection of resistance to each antibiotic delineated by horizontal lines. The lower, middle, and upper sub-catchments are delineated by dotted vertical lines at 52 km and 106 km. Sample sites denoted by small vertical lines directly above the x-axis of each plot.

There were significantly higher ($p < 0.10$) levels of ARB in the water column of the lower sub-catchment and coliform bacteria were 2.3 times more likely to express phenotypic resistance than *E. coli* (Fig. 2-3A). The probability of AR coliforms was three times higher in tributaries than in the main stem of the middle and lower sub-catchments (Fig. 2-3B). In the upper sub-catchment, bacterial resistance was similar in each stream compartment (water versus biofilm) and stream type (main stem versus tributary).

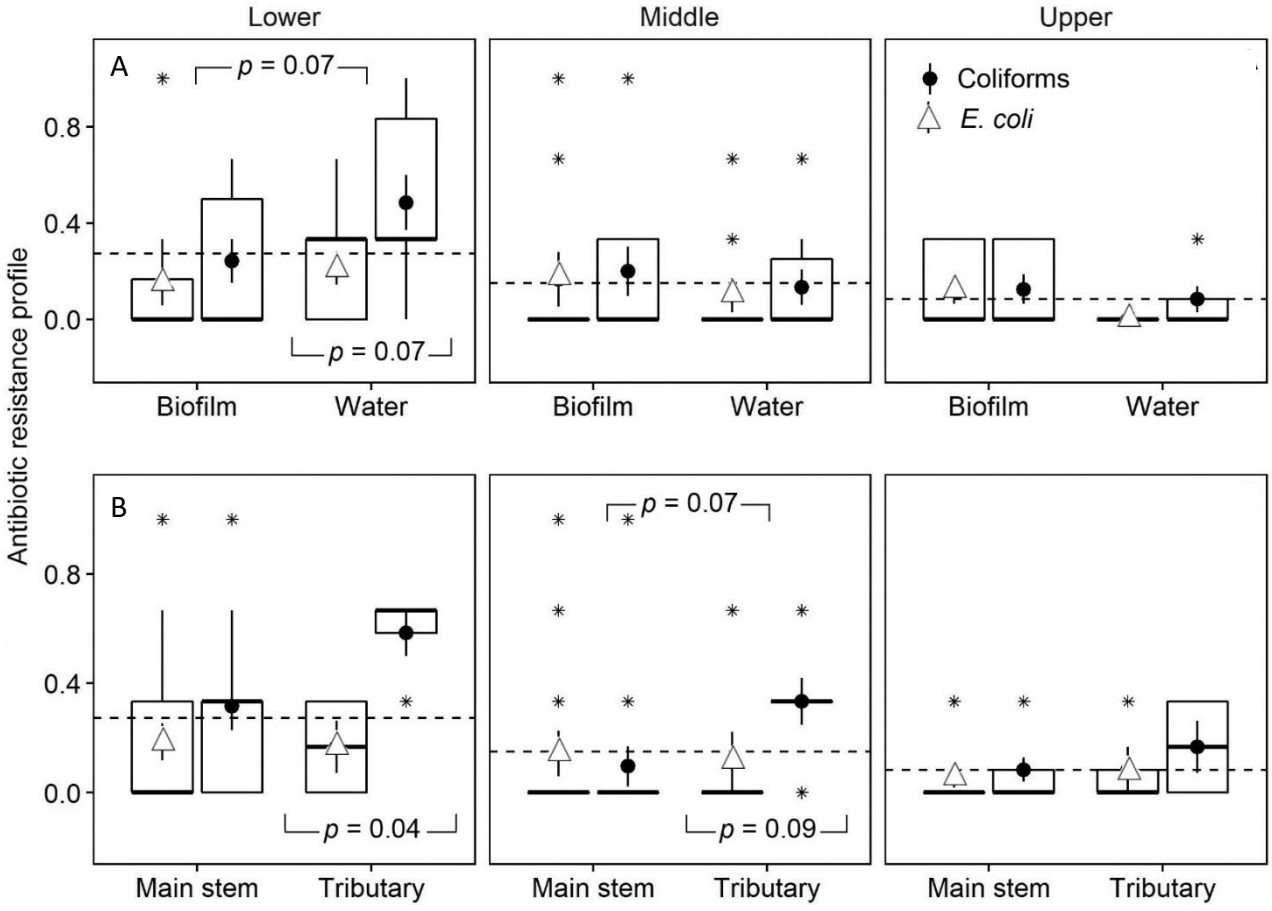


Figure 2-3: Modeled profile of antibiotic resistance for *E. coli* and coliform bacteria in (A) water column and biofilms, and (B) in the main stem and tributaries in the lower, middle, and upper sub-catchments. Statistically significant differences ($p < 0.10$) of pair-wise comparisons based on Wilcoxon rank-sum tests are reported above bars for differences between factor levels and below bars for differences by metabolism. Symbols represent the standard error of the mean (SEM) with the grand mean across each sub-catchment shown as a horizontal dotted line.

3.3. Antibiotic resistance to individual antibiotics

Proportions of bacterial samples with phenotypic resistance to individual antibiotics were highest for resistance to ampicillin (Amp^r) 29% (34 / 116) followed by resistance to tetracycline (Tet^r) 15% (17 / 116) and resistance to chloramphenicol (Cm^r) 10% (11 / 116). There were statistically significant differences between resistance to individual antibiotics (X^2 [df = 2, N = 348] = 16.76, $p < 0.001$) with significantly higher occurrence of Amp^r to Cm^r (Tukey multiple comparison of means, $p < 0.001$) and Amp^r to Tet^r ($p < 0.01$). The proportions of bacteria Tet^r to Cm^r were similar.

Antibiotic resistance traits were uniquely distributed by stream compartment, stream type, and longitudinal distance. There was higher probability of detecting resistance traits in tthe water column of tributaries lities of Kernel density mapping of resistance traits

3.4. Generalized linear regressions of antibiotic resistance

The probability of antibiotic resistance was positively associated with the CV of environmental variability, inability to ferment lactose, and tributaries (Table 2-4, Fig. 2-4). The rank of the antibiotic resistance profile was positively associated with higher environmental variability and probability of detection in tributaries, but there were non-significant differences between lactose fermenters and non-lactose fermenters. For both models, the differences between the sub-catchments explained ~2 times the variance of the fixed effects (Table 2-4).

Table 2-4: Parameter estimates and boot-strapped 95% confidence intervals of the best generalized linear mixed models for the detection of antibiotic resistance (binomial) and the antibiotic resistance profile (negative binomial) with the fixed effects conditional on the random effect.

Model	Parameter	Estimate ± SEM	95% CI		Marginal R^2 †	Conditional R^2 †
			Lower	Upper		
Binomial	Intercept	-3.50 ± 0.52	-4.88	-2.61	0.22[a]	0.39[a]
	CV	0.71 ± 0.25	0.23	1.32		
	Tributary	1.13 ± 0.51	0.08	2.34		
	Coliforms	0.81 ± 0.33	0.22	1.47		
	Amp^r	1.67 ± 0.42	0.91	2.63		
Negative binomial	Intercept	-2.48 ± 0.35	-3.25	-1.92	0.13[a]	0.27[a]
	CV	0.65 ± 0.23	0.29	1.20		
	Tributary	1.02 ± 0.45	-0.09	1.99		
	Coliforms	0.73 ± 0.31	0.09	1.42		

† R^2 values calculated to indicate the proportion of variance explained by the fixed effects (marginal R^2; CV, stream type, metabolism, Amp^r) for the fixed and random effects (conditional R^2: including differences between the upper, middle, and lower sub-catchments).

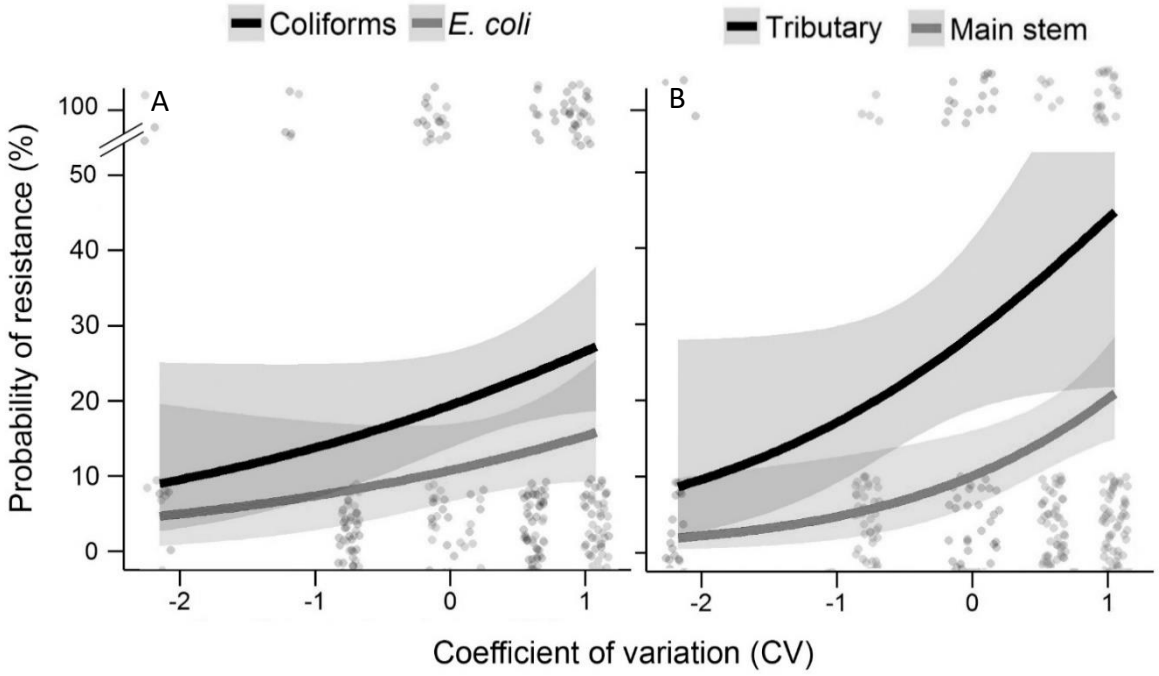


Figure 2-4: Predicted probability (lines) with 95% confidence intervals of the best model for the detection of antibiotic resistance. Lines represent the fixed effects of increasing summed coefficient of variation (CV) for water temperature, specific conductance, stream power, and stream sinuosity based on the random effect of sampling sites nested within sub-catchment. The plot on the left shows *E. coli* (grey line) and coliforms (black line), and plot on the right shows the main stem (grey line) and tributaries (black line). Sample points for each are jittered graphically to facilitate visualization of the occurrence patterns and CV was centered and scaled.

4. Discussion

Understanding how natural microbial communities respond to abiotic and biotic inputs from human activity has implications for freshwater ecosystems and human health. This is a new frontier in ecological and environmental science (Rosi et al., 2018b) driven by recent developments in analytical approaches. We employed a holistic riverscape framework linking physical, chemical, and biological impacts to quantify spatial patterns of bacterial antibiotic resistance and to elucidate

potential drivers of these patterns. We examined the spatial distribution of AR Gram-negative endogenous coliforms and exogenous *E. coli* in relation to environmental and ecological variables, and ubiquitous non-point source pollution. The distribution of *E. coli* was shown to be useful ecological indicators because these organisms are commonly monitored and have been found to influence the structure of natural bacterial communities (Ramirez et al., 2018). We hypothesized that the spatial patterns of ARB would vary with environmental conditions, and therefore we applied macro-ecology principles at the spatial scales relevant to municipal resource managers (Barber et al., 2014; Lennon & Locey, 2016).

This study revealed extensive contamination by ARB across the entire river network with distinct areas of clearing immediately downstream of green belts. Urban development was strongly associated with antibiotic resistance among endogenous bacteria in tributaries suggesting that anthropogenic non-point source pollution may disproportionately transform natural bacteria to more resistant forms in smaller streams which then accumulate downstream in the water column. Antibiotic resistant *E. coli* were heterogeneously distributed across the basin which may be due to the wide-spread impact of pollutants continuously entering the river along its entire length. Further studies are needed to evaluate how conditions in urban rivers which lack wastewater discharges contribute to the occurrence of ARB and the associated risks to human, animal, and environmental health. The trait for metabolism of lactose was utilized to quickly differentiate naturally occurring fecal coliforms arising from wild animals from exogenous bacteria such as *E. coli* which is an indicator of human and farm animal fecal contamination. Lactose metabolism is a distinctive trait that reflects an evolutionary history that comprises the essential determinants of fitness within the primary ecology of the organism (Plata et al., 2015). Therefore, the concepts of macroecology can be applied to study riverine bacteria as traits for antibiotic resistance are frequently an adaptive response to environmental conditions. Barriers persist in the application of these principles to bacterial

ecology because micro-organisms evolve rapidly, share traits across taxa, are incredibly diverse, and exhibit high dispersal capacity particularly in flowing water. Thus, approaches that integrate macro-ecological concepts to bacterial community structure have rarely been applied to assess the ecological health and management of the urban river. Problems of spatial scale and directional flow further complicate the study of microbiology across riverscapes (Barberan et al., 2014). In spite of these obstacles, macro-ecological drivers have been identified as the primary determinants of bacterial distributions across multiple ecological domains and spatial scales including deep marine benthic environments (Danovaro et al., 2016), open ocean waters (Amend et al., 2013), and soils (Hendershot et al., 2017).

We found that environmental variability was positively correlated with basin-wide urban development and pollutant inputs, and although AR coliforms and *E. coli* were associated with urbanization and detected at 90% of sites, there was discontinuity in their respective distributions. For example, ARB were found in both polluted and pristine river segments, but their prevalence in the most polluted lower sub-catchment differed by lactose metabolism and mode of life (e.g., bacteria whose habitat was the water column), whereas in the middle sub-catchment differences of lactose metabolism and stream type predominated. These results support the use of a holistic framework to predict the distribution of antibiotic resistance across urban river systems with no major point sources of pollutants. By assessing the dynamics of bacterial antibiotic resistance within the context of landscape macroecological concepts we were able to identify the unique spatial patterns of antibiotic resistance among endogenous and exogenous bacteria across the basin.

4.1. Antibiotic resistant bacteria and disturbance

In this study, ARB were detected in both heavily developed areas and sites with little human activity. However, there were notable exceptions to this spatial pattern. There was no ARB detected

along two river segments that traversed green belts within intensive urban development upstream and downstream. Additionally, Tet^r and Chlor^r were not detected upstream of the Howard Hansen Reservoir (Fig. 2-5). This indicates that the patterns of ARB are responsive to the conditions at the river segment, reach, or pool-riffle-run scale. Furthermore, the distinct differences between antibiotic resistant water-borne bacteria and biofilms in highly developed areas indicated that sampling the water column may not capture the prevalence of ARB in areas that commonly exceed water quality standards. This is an important aspect of monitoring because sediment-bound contaminants that include ARB are re-suspended after precipitation events. The difference between the antibiotic resistance profile of lactose fermenting and non-lactose fermenting bacteria by sub-catchment suggests an ecological response to land use.

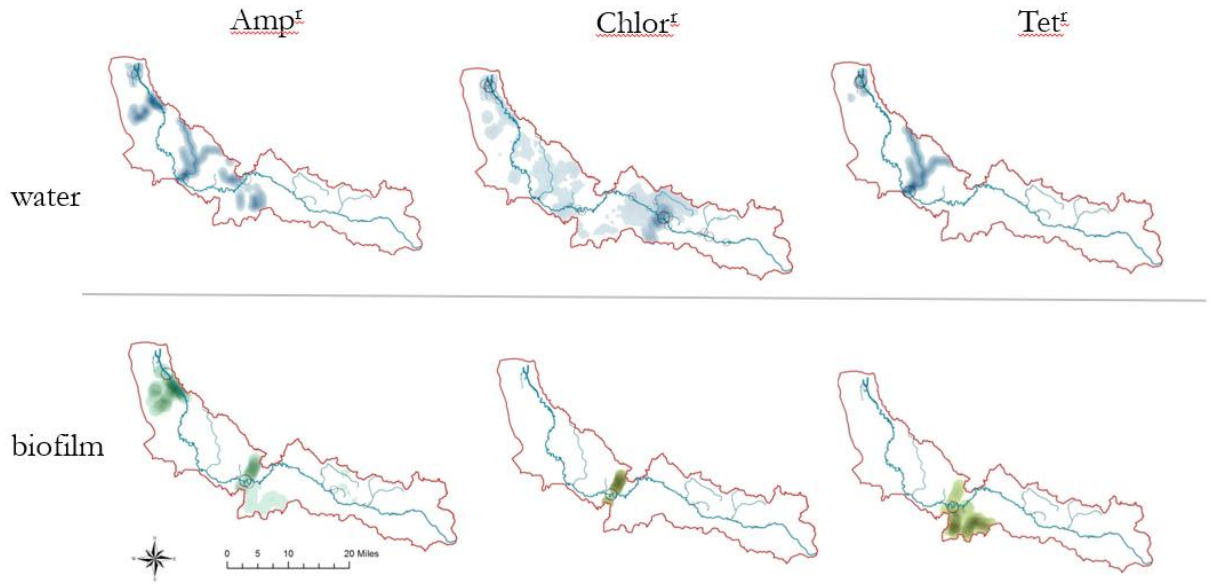


Figure 2-5: Kernel density mapping of the best model mixed effects model for the detection of antibiotic resistance. The maps show higher probabilities in the water column of tributaries for Amp^r and Tet^r, whereas, Chlor^r was less likely to be detected across the entire watershed and not closely associated with any particular tributary. Resistance in biofilms indicated different probabilities and were closely associated specific tributaries draining agricultural areas in the middle sub-catchment and extensively polluted river segments in the lower sub-catchment.

Resistance to ampicillin was the most detected resistance factor in our study and contributed most to the proportions of ARB in each sub-catchment. In the upper sub-catchment, Amp_r was detected in 25% of samples (8/32) and was the only resistance factor detected downstream for 67 km. This may be the natural background level of resistance or a legacy effect of the numerous small mining and logging towns that existed in the headwaters prior to 1920 (Freeman et al., 2007). We detected resistance by sampling bacterial communities, and thus, it was not possible to identify co-resistance among individual bacterial species, but there was only one instance where Tet^r was not associated with Amp^r, and Cm^r was always associated with either Amp^r or Tet^r.

The average MPN of total coliform was highest in the Howard Hansen Reservoir, most likely due to the longer water residence time and substantial numbers of waterfowl and native cervids in the area. In contrast, the average *E. coli* MPN was highest in the lower sub-catchment where pollutants and bacteria are likely to accumulate. In the middle sub-catchment, coliform and *E. coli* MPN were significantly lower (75%) than the upper and lower sub-catchments. The middle sub-catchment has been minimally modified by human activities and has largely natural riparian structure and geomorphology. The Washington State Department of Ecology 1998 303(d) list of impaired waterways found no exceedance of pollutants other than fecal coliform and increased temperature in the main stem in this catchment. Although relatively pristine environmental conditions predominated in the middle sub-catchment, there was more ARB detected in the tributaries than in the main stem. This suggested that sub-catchment size may be a driver for higher levels of resistance even when there was less impact from land development. Environmental variability increased with development in both stream types, but tributaries consistently contained higher levels of ARB than the mainstem.

The broad spectrum of environmental conditions and microbial communities may have contributed to the prevalence and distribution of antibiotic resistant non-*E. coli* coliforms in the

streams that we sampled. This indicates a trend of increasing antibiotic resistance at two levels of organization: (1) an overall increase of ARB with environmental variability, and probable accumulation downstream in the main stem; and (2) higher antibiotic resistance among non-lactose fermenting bacteria under more stable conditions in tributaries. This is consistent with what has previously been found in the Pacific Northwest (USA) where water-associated bacteria were found to respond to large-scale environmental gradients, but ecological factors were the primary drivers of processes at smaller scales (Fortunato & Crump, 2011).

One function of antibiotic resistance is the evolutionary response to survive in deteriorating environments (Davies, 2006; González-Zorn & Escudero, 2012). Although significant positive correlations exist between environmental ARB and urban development, our findings corroborate other studies showing that the spatial patterns of ARB differ due to ecological processes that vary with scale (McArthur & Tuckfield, 2000). Antibiotic resistant bacteria were detected at most sites, but resistant coliforms were more prevalent and demonstrated higher levels of resistance (antibiotic resistance profile) than *E. coli*. Research supports that land use increases antibiotic resistance among both endogenous and exogenous bacteria, but ultimately survival in human-dominated waterways requires tradeoffs between fitness costs and survival benefits that are dependent upon local conditions (Gifford et al., 2016; Marti et al., 2017; Poff, 2014).

4.2. Patterns of antibiotic resistance and water-borne bacterial counts

The Washington State Department of Ecology lists four segments on the Green-Duwamish River under the Clean Water Act Section 303(d) as impaired for exceeding fecal bacteria concentrations. These segments begin near river km 90 and occur in several reaches downstream to the mouth of the river (www.ecology.wa.gov). We found that the antibiotic resistance profile of coliform bacteria in the water column nearly doubled between the middle and lower sub-

catchments, and this may indicate a rapid response to abrupt environmental change or simply accumulation from downstream flow. The inferences drawn from the spatial patterns of resistant bacteria may differ based on the bacterial groups targeted for monitoring by regulatory agencies (i.e., *E. coli, Salmonella* or *Enterococci*) (McArthur et al., 2016). These species are often monitored and most likely vary individually according to the scale of the sampling across various environmental gradients. Therefore, analysis of pooled samples at large spatial scales will likely overwhelm the influence of any factors at the reach scale. Boon & Cattanach (1999) found that coliform bacteria comprise a higher proportion of samples and on average are two times more likely to be antibiotic resistant compared to *E. coli*. Similarly, we detected higher proportions of antibiotic resistant coliforms in every sample, including the most degraded reaches, and their level of resistance increased exponentially downstream. There can be a fitness cost to the carriage of ARGs when no selective pressure is present, as is evidenced by reduced growth rates and competitive ability (Andersson & Hughes, 2010). Thus, detection of resistant coliforms may represent a robust ecologically mediated fitness and ability to exploit a wider variety of niches than bacteria from human sources. In our study, *E. coli* was less prevalent and had a patchy distribution across the catchment, an indication of widespread non-point sources of exogenous fecal bacteria.

There were two segments on the main stem where clearing of ABR occurred (i.e., ABR not detected immediately downstream from a detection site): (1) a 12-km segment (river km 91-103) that included two state parks where ARB were detected immediately upstream and downstream of two small communities; and (2) a segment adjacent to a small state conservation area (river km 55.4). This green belt had an intact riparian zone, and the river channel was highly incised and tortuous. The state park divided areas of intensive upstream and downstream bankside residential development. Together, the unique conditions in these two segments appeared to mitigate the prevalence of ARB. Although these were the only sites without detectable ARB, a finer level of

sampling may have allowed us to detect spatial patterns and identify reach-scale clearing. The relatively consistent conditions in the middle and lower sub-catchment main stem may explain why antibiotic resistance generally increased downstream but remained similar between coliforms and *E. coli*.

Higher levels of antibiotic resistant Gram-negative coliforms in tributaries may be related to terrestrial sources: (1) intrinsically resistant bacteria originating from soils, (2) wild and domestic animal-based bacteria that have acquired resistance (Boon & Cattanach, 1999), and (3) shallow and/or failing septic systems (Whitlock et al., 2002). Hydrological connectedness between the tributaries and the main stem is likely a primary mechanism by which ARB and ARGs accumulate downstream (Verhougstraete et al., 2015). The unique environmental and ecological factors present in the low-order streams may explain why coliforms have higher levels of antibiotic resistance even though conditions were more stable. This observation was supported by our GLMM results which indicated that stream type was a strong driver of antibiotic resistance.

The tributaries in the lower sub-catchment drained the fully developed residential and industrial areas. The soils in these drainage basins were highly contaminated from industrial development dating from the early 1900s, and untreated sewage was discharged into the middle and lower sub-catchments at Auburn, Kent, and Renton, Washington until 1987 (https://www.historylink.org/File/11250). The legacy of contamination from industrial and waste-water effluents can alter ecological function for decades (Bradley et al., 2016). The Green-Duwamish is a Superfund Site that poses a human health risk from polychlorinated biphenyls, arsenic, carcinogenic polycyclic aromatic hydrocarbons, heavy metals as well as dioxins and furans (EPA, 2019). Many of these contaminants are antibiotic resistant determinants that increase the level of ARB, particularly in biofilms (Abraham, 2011; Hosen et al., 2017). Furthermore, tributary flow paths often parallel roads in highly developed areas which increases the likelihood of contamination from

transportation-based heavy metals, toxins, solvents, petroleum products, and exogenous bacteria in runoff.

Abiotic and biotic conditions from both natural riverine processes and the selective pressure of development alter the structure and function of bacterial communities. The complex nature of human-induced environmental disturbance and the health of urban rivers remain poorly understood (Craig et al., 2017). These interactions vary over space and time and comprise the primary driver of the abundance and diversity of ARGs (Proia et al., 2013; Storteboom et al., 2010; Washington et al., 2013). Tributary confluences create physicochemical discontinuities that influence bacterial communities (Beier et al., 2008; Benda et al., 2004; Winter et al., 2007). Tributary-specific factors may influence the spatial patterns of ARB and ARGs in the main stem, and this ultimately determines the diversity and prevalence of ARB in downstream segments.

Land development influences stream ecosystems, and natural selection will continue to drive bacterial response. Due to the global increase in bacterial resistance, natural resource managers may need to mitigate the proliferation of ARB by interrupting the flow of resistance genes through the urban water cycle. Although knowledge of ARB in riverine systems is improving, most studies are conducted in a spatially limited manner in relation to point sources (Moore et al., 2010; Zhou et al., 2017). Spatially explicit assessments that encompass the physical, chemical, and ecological dynamics of the urban riverscape (*sensu* Fausch et al., 2002) provide a more holistic framework for discerning trends in bacterial resistance throughout the river continuum. Urban rivers provide unique opportunities for the diversification and circulation of bacterial traits ecological systems. Given that urban rivers are significant resources for humans and non-human animals, baseline surveys of bacterial traits may inform efforts to decrease health risks and monitor human impacts. Comparing the spatial patterns of AR exogenous and endogenous bacteria sheds light on how environmental conditions drive the prevalence and fate of antibiotic resistance traits in bacterial communities and

may better guide mitigation efforts toward a holistic view of riverscape microbiology that acknowledges antibiotic resistance as traits subject to Darwinian natural selection within the full context of bacterial life.

Acknowledgements

We received financial support from the Xi Sigma Pi Forestry Honors Society, Alpha Chapter at the University of Washington, and the U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center. We thank Heidi Gough and Carrie Givens for their constructive comments on early versions of this manuscript, and the City of Tacoma, Department of Public Utilities for logistical support and assistance with sampling in the upper sub-catchment. In addition, Rory Denovan from Seattle City Light provided invaluable assistance during field work in the lower Duwamish. This work could not have been completed without the help provided by Marc Klock, Taylor Klock, and Travis Pedersen. We also thank David No for help with bacteriological identification and assessment. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

References

Abraham, W. R. (2011). Megacities as sources for pathogenic bacteria in rivers and their fate downstream. *International Journal of Microbiology*, *2011,*(Article ID 798292). https://doi.org/10.1155/2011/798292

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K., Davies, J., & Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, *8*(4), 251–259. https://doi.org/10.1038/nrmicro2312

Almakki, A., Jumas-Bilak, E., Marchandin, H., & Licznar-Fajardo, P. (2019). Antibiotic resistance in urban runoff. *Science of the Total Environment*, *667*, 64–76. https://doi.org/10.1016/j.scitotenv.2019.02.183

Amend, A. S., Oliver, T. A., Amaral-Zettler, L. A., Boetius, A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., Martiny, A. C., Ramette, A., Zinger, L., Sogin, M. L., & Martiny, J. B. H. (2013). Macroecological patterns of marine bacteria on a global scale. *Journal of Biogeography*, *40*(4), 800–811. https://doi.org/10.1111/jbi.12034

Amos, G. C., Gozzard, E., Carter, C. E., Mead, A., Bowes, M. J., Hawkey, P. M., Zhang, L., Singer, A. C., Gaze, W. H., & Wellington, E. M. (2015). Validated predictive modelling of the environmental resistome. *The ISME Journal*, *9*(6), 1467–1476. https://doi.org/10.1038/ismej.2014.237

Andersson, D. I., & Hughes, D. (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews Microbiology*, *8*(4), 260–271. https://doi.org/10.1038/nrmicro2319

Ash, R. J., Mauck, B., & Morgan, M. (2002). Antibiotic resistance of Gram-negative bacteria in rivers, United States. *Emerging Infectious Diseases*, *8*(7), 713–716.

Barberan, A., Casamayor, E. O., & Fierer, N. (2014). The microbial contribution to macroecology. *Frontiers in Microbiology*, 1–8. https://doi.org/10.3389/fmicb.2014.00203

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beier, S., Witzel, K.-P., & Marxsen, J. (2008). Bacterial community composition in central european running waters examined by temperature gradient gel electrophoresis and sequence analysis of 16S rRNA genes. *Applied and Environmental Microbiology*, *74*(1), 188–199. https://doi.org/10.1128/AEM.00327-07

Benda, L., Andras, K., Miller, D., & Bigelow, P. (2004). Confluence effects in rivers: Interactions of basin scale, network geometry, and disturbance regimes. *Water Resources Research*, *40*(5), 1–15. https://doi.org/10.1029/2003WR002583

Bernhardt, E. S., & Palmer, M. A. (2007). Restoring streams in an urbanizing world. *Freshwater Biology*, *52*(4), 738–751. https://doi.org/10.1111/j.1365-2427.2006.01718.x

Besemer, K., Peter, H., Logue, J. B., Langenheder, S., Lindström, E. S., Tranvik, L. J., & Battin, T. J. (2012). Unraveling assembly of stream biofilm communities. *The ISME Journal*, *6*(8), 1459–1468. https://doi.org/10.1038/ismej.2011.205

Besemer, Katharina, Singer, G., Hödl, I., & Battin, T. J. (2009). Bacterial Community Composition of Stream Biofilms in Spatially Variable-Flow Environments. *Applied and Environmental Microbiology*, *75*(22), 7189–7195. https://doi.org/10.1128/AEM.01284-09

Boon, P. I., & Cattanach, M. (1999). Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, south-eastern Australia. *Letters in Applied Microbiology*, *28*(3), 164–168. https://doi.org/10.1046/j.1365-2672.1999.00517.x

Bradley, P. M., Barber, L. B., Clark, J. M., Duris, J. W., Foreman, W. T., Furlong, E. T., Givens, C. E., Hubbard, L. E., Hutchinson, K. J., Journey, C. A., Keefe, S. H., & Kolpin, D. W. (2016). Pre/post-closure assessment of groundwater pharmaceutical fate in a wastewater-facility-impacted stream reach. *Science of the Total Environment*, *568*, 916–925. https://doi.org/10.1016/j.scitotenv.2016.06.104

Brendel, C., & Soupir, M. L. (2017). Relating watershed characteristics to elevated stream *Escherichia coli* levels in agriculturally dominated landscapes: An Iowa case study. *Water*, *9*(154). https://doi.org/10.3390/w9030154

Craig, L. S., Olden, J. D., Arthington, A. H., Entrekin, S., Hawkins, C. P., Kelly, J. J., Kennedy, T. A., Maitland, B. M., Rosi, E. J., Roy, A. H., Strayer, D. L., Tank, J. L., West, A. O., & Wooten, M. S. (2017). Meeting the challenge of interacting threats in freshwater ecosystems: A call to scientists and managers. *Elem Sci Anth*, *5*(0), 72. https://doi.org/10.1525/elementa.256

Crump, B. C., Amaral-Zettler, L. A., & Kling, G. W. (2012). Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME Journal*, *6*(9), 1629–1639. https://doi.org/10.1038/ismej.2012.9

Danovaro, R., Molari, M., Corinaldesi, C., & Dell'Anno, A. (2016). Macroecological drivers of archaea and bacteria in benthic deep-sea ecosystems. *Science Advances*, *2*(4), 1–12. https://doi.org/10.1126/sciadv.1500961

Davies, J. (2006). Are antibiotics naturally antibiotics? *Journal of Industrial Microbiology and Biotechnology*, *33*(7), 496–499. https://doi.org/10.1007/s10295-006-0112-5

Department of Ecology. (2014). *River and stream water quality monitoring report water year 2013 (Issue Publication No. 14-03-047)*. Washington Department of Ecology, Olympia, WA, USA. https://fortress.wa.gov/ecy/publications/SummaryPages/1403047.html%0AEcology's

De Souza, M. J., Nair, S., Loka Bharathi, P. A., & Chandramohan, D. (2006). Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic marine waters. *Ecotoxicology*, *15*(4), 379–384. https://doi.org/10.1007/s10646-006-0068-2

Easthouse, K. (2008). Howard Hanson Dam: Green River and Howard Hanson Reservoir water quality sampling and analysis plan 2008. http://www.nws.usace.army.mil/

Edwards, R. T., Meyer, J. L., & Findlay, S. E. G. (1990). The relative contribution of benthic and suspended bacteria to system biomass, production, and metabolism in a low-gradient blackwater river. *Journal of the North American Benthological Society*, *9*(3), 216–228.

Environmental Protection Agency (EPA). (2019). *Lower Duwamish Waterway Seattle, WA. Contaminant List*. https://cumulis.epa.gov/supercpad/SiteProfiles/index.cfm?fuseaction=second.contams &id =1002020

Environmental Systems Research Institute (ESRI). (2014). *ArcGIS Desktop: Release 10.1* (ArcGIS 10.1 Environmental Systems Research Institute). Environmental Systems Research Institute.

Fani, R. (2012). The Origin and Evolution of Metabolic Pathways: Why and How did Primordial Cells Construct Metabolic Routes? *Evolution: Education and Outreach*, *5*(3), 367–381. https://doi.org/10.1007/s12052-012-0439-5

Fausch, K. D., Torgersen, C. E., Baxter, C. V, & Li, H. W. (2002). Landscapes to riverscapes: Bridging the gap between research and conservation of stream fishes. *BioScience*, *52*(6), 483–498. https://doi.org/10.1641/0006-3568(2002)052[0483:LTRBTG]2.0.CO;2

Feltz, C. J., & Miller, G. E. (1996). An asymptotic test for the equality of coefficients of variation from k populations. *Statistics in Medicine*, *15*(6), 647–658. https://doi.org/10.1002/(SICI)1097-0258(19960330)15:6<647::AID-SIM184>3.0.CO;2-P

Ferguson, R. I., Cudden, J. R., Hoey, T. B., & Rice, S. P. (2006). River system discontinuities due to lateral inputs: Generic styles and controls. *Earth Surface Processes and Landforms*, *31*(9), 1149–1166. https://doi.org/10.1002/esp.1309

Findlay, R. H., Yeates, C., Hullar, M. A. J., Stahl, D. A., & Kaplan, L. A. (2008). Biome-level biogeography of streambed microbiota. *Applied and Environmental Microbiology*, *74*(10), 3014–3021. https://doi.org/10.1128/AEM.01809-07

Flemming, H., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94

Fletcher, M. (1992). Bacterial metabolism in biofilms. In L. Melo, T. R. Bott, & B. Capdeville (Eds.), *Biofilms—science and technology* (pp. 113–124). Dordrecht: Kluwer Academic Publishers. https://doi.org/10.1007/978-94-011-1824-8_12

Fortunato, C. S., & Crump, B. C. (2011). Bacterioplankton community variation across river to ocean environmental gradients. *Microbial Ecology*, *62*(2), 374–382. https://doi.org/10.1007/s00248-011-9805-z

Freeman, M. C., Pringle, C. M., & Jackson, C. R. (2007). Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *Journal of the American Water Resources Association*, *43*(1), 5–14. https://doi.org/10.1111/j.1752-1688.2007.00002.x

Garcia-Armisen, T., Vercammen, K., Passerat, J., Triest, D., Servais, P., & Cornelis, P. (2011). Antimicrobial resistance of heterotrophic bacteria in sewage-contaminated rivers. *Water Research*, *45*(2), 788–796. https://doi.org/10.1016/j.watres.2010.09.003

Gifford, D. R., Moss, E., & Maclean, R. C. (2016). Environmental variation alters the fitness effects of rifampicin resistance mutations in *Pseudomonas aeruginosa*. *Evolution*, *70*(3), 725–730. https://doi.org/10.1111/evo.12880

González-Zorn, B., & Escudero, J. A. (2012). Ecology of antimicrobial resistance: humans, animals, food and environment. *International Microbiology*, *15*, 101–109. https://doi.org/10.2436/20.1501.01.163

Hendershot, J. N., Read, Q. D., Henning, J. A., Sanders, N. J., & Classen, A. T. (2017). Consistently inconsistent drivers of microbial diversity and abundance at macroecological scales. *Ecology*, *98*(7), 1757–1763. https://doi.org/10.1002/ecy.1829

Hosen, J. D., Febria, C. M., Crump, B. C., & Palmer, M. A. (2017). Watershed urbanization linked to differences in stream bacterial community composition. *Frontiers in Microbiology*, *8*(AUG), 1–17. https://doi.org/10.3389/fmicb.2017.01452

Karkman, A., Pärnänen, K., & Larsson, D. G. J. (2019). Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. *Nature Communications*, *10*(1), 1–8. https://doi.org/10.1038/s41467-018-07992-3

Keen, P. L., & Patrick, D. M. (2013). Tracking Change: A look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics*, *2*, 191–205. https://doi.org/10.3390/antibiotics2020191

King County Department of Natural Resources. (2000). *Habitat limiting factors and reconnaissance assessment report* (Issue December). http://www.govlink.org/watersheds/9/reports/Recon.aspx

Kreimer, A., Borenstein, E., Gophna, U., & Ruppin, E. (2008). The evolution of modularity in bacterial metabolic networks. *Proceedings of the National Academy of Sciences*, *105*(19), 6976–6981. https://doi.org/10.1073/pnas.0712149105

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology*, *46*(1), 165–170. https://doi.org/10.1007/s11356-014-3887-3

Leff, L. G., McArthur, J. V., & Shimkets, L. J. (1992). Information spiraling: movement of bacteria and their genes in streams. *Microbial Ecology*, *24*, 11–24.

Lennon, J. T., & Locey, K. J. (2016). Macroecology for microbiology. *Environmental Microbiology Reports*, *9*(1), 38–40. https://doi.org/10.1111/1758-2229.12512

MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M., & Brownstein, J. S. (2018). Antibiotic resistance increases with local temperature. *Nature Climate Change*, *8*, 510–514. https://doi.org/10.1038/s41558-018-0161-6

Marti, R., Ribun, S., Aubin, J. B., Colinon, C., Petit, S., Marjolet, L., Gourmelon, M., Schmitt, L., Breil, P., Cottet, M., & Cournoyer, B. (2017). Human-driven microbiological contamination of benthic and hyporheic sediments of an intermittent peri-urban river assessed from MST and 16S rRNA genetic structure analyses. *Frontiers in Microbiology*, *8*, 1–15. https://doi.org/10.3389/fmicb.2017.00019

Martinez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution*, *157*(11), 2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Martinez, J. L. (2012). Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. *Frontiers in Microbiology*, *2*(JAN), 1–6. https://doi.org/10.3389/fmicb.2011.00265

Martinez, Jose L. (2014). General principles of antibiotic resistance in bacteria. *Drug Discovery Today: Technologies*, *11*(1), 33–39. https://doi.org/10.1016/j.ddtec.2014.02.001

McArthur, J. V., Fletcher, D. E., Tuckfield, R. C., & Baker-Austin, C. (2016). Patterns of multi-antibiotic-resistant *Escherichia coli* from streams with no history of antimicrobial inputs. *Microbial Ecology*, *72*(4), 840–850. https://doi.org/10.1007/s00248-015-0678-4

McArthur, J. V., & Tuckfield, R. C. (2000). Spatial patterns in antibiotic resistance among stream bacteria: effects of industrial pollution. *Applied and Environmental Microbiology*, *66*(9), 3722–3726.

McGill, B. J. (2019). The what, how and why of doing macroecology. *Global Ecology and Biogeography*, *28*(1), 6–17. https://doi.org/10.1111/geb.12855

Moore, J. E., Rao, J. R., Moore, P. J. A., Millar, B. C., Goldsmith, C. E., Loughrey, A., & Rooney, P. J. (2010). Determination of total antibiotic resistance in waterborne bacteria in rivers and streams in Northern Ireland: Can antibiotic-resistant bacteria be an indicator of ecological change? *Aquatic Ecology*, *44*(2), 349–358. https://doi.org/10.1007/s10452-009-9294-z

Nazaret, S., & Aminov, R. (2014). Role and prevalence of antibiosis and the related resistance genes in the environment. *Frontiers in Microbiology (5)*Sep. https://doi.org/10.3389/fmicb.2014.00520

Plata, G., Henry, C. S., & Vitkup, D. (2015). Long-term phenotypic evolution of bacteria. *Nature*, *517*(7534), 369–372. https://doi.org/10.1038/nature13827

Poff, N. L. (2014). Rivers of the anthropocene? *Frontiers in Ecology and the Environment*, *12*(8), 427. https://doi.org/10.1890/1540-9295-12.8.427

Pontes, D. S., Pinheiro, F. A., Lima-Bittencourt, C. I., Guedes, R. L. M., Cursino, L., Barbosa, F., Santos, F. R., Chartone-Souza, E., & Nascimento, A. M. A. (2009). Multiple antimicrobial resistance of Gram-negative bacteria from natural oligotrophic lakes under distinct anthropogenic influence in a tropical region. *Microbial Ecology*, *58*(4), 762–772. https://doi.org/10.1007/s00248-009-9539-3

Proia, L., Lupini, G., Osorio, V., Pérez, S., Barceló, D., Schwartz, T., Amalfitano, S., Fazi, S., Romaní, M., & Sabater, S. (2013). Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river. *Chemosphere*, *92*(9), 1126–1135. https://doi.org/10.1016/j.chemosphere.2013.01.063

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

Ramirez, K. S., Knight, C. G., De Hollander, M., Brearley, F. Q., Constantinides, B., Cotton, A., Creer, S., Crowther, T. W., Davison, J., Delgado-Baquerizo, M., Dorrepaal, E., Elliott, D. R., Fox, G., Griffiths, R. I., Hale, C., Hartman, K., Houlden, A., Jones, D. L., Krab, E. J., … De Vries, F. T. (2018). Detecting macroecological patterns in bacterial communities across independent studies of global soils. *Nature Microbiology*, *3*(2), 189–196. https://doi.org/10.1038/s41564-017-0062-x

Roger, F., Caron, A., Morand, S., Pedrono, M., Garine-Wichatitsky, M. de, Chevalier, V., Tran, A., Gaidet, N., Figuié, M., de Visscher, M.-N., & Binot, A. (2016). One Health and EcoHealth: the same wine in different bottles? *Infection Ecology & Epidemiology*, *6*(1), 30978. https://doi.org/10.3402/iee.v6.30978

Rompré, A., Servais, P., Baudart, J., De-Roubin, M. R., & Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *Journal of Microbiological Methods*, *49*(1), 31–54. http://www.ncbi.nlm.nih.gov/pubmed/11777581

Rosi-Marshall, E. J., Snow, D., Bartelt-Hunt, S. L., Paspalof, A., & Tank, J. L. (2015). A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. *Journal of Hazardous Materials*, *282*, 18–25. https://doi.org/10.1016/j.jhazmat.2014.06.062

Rosi, E. J., Bechtold, H. A., Snow, D., Rojas, M., Reisinger, A. J., & Kelly, J. J. (2018a). Urban stream microbial communities show resistance to pharmaceutical exposure. *Ecosphere*, *9*(1). https://doi.org/10.1002/ecs2.2041

Rosi, E. J., Bechtold, H. A., Snow, D., Rojas, M., Reisinger, A. J., & Kelly, J. J. (2018b). Urban stream microbial communities show resistance to pharmaceutical exposure. *Ecosphere*, *9*(1), 1–16. https://doi.org/10.1002/ecs2.2041

Rousham, E. K., Unicomb, L., & Islam, M. A. (2018). Human, animal and environmental contributors to antibiotic resistance in low-resource settings: Integrating behavioural, epidemiological and one health approaches. *Proceedings of the Royal Society B: Biological Sciences*, *285*. https://doi.org/10.1098/rspb.2018.0332

Sanderson, C. E., Fox, J. T., Dougherty, E. R., Cameron, A. D. S., & Alexander, K. A. (2018). The changing face of water: A dynamic reflection of antibiotic resistance across landscapes. *Frontiers in Microbiology*, *9*(1894), 1–13. https://doi.org/10.3389/fmicb.2018.01894

Singer, R. S., Ward, M. P., & Maldonado, G. (2006). Can landscape ecology untangle the complexity of antibiotic resistance? *Nature Reviews. Microbiology*, *4*(12), 943–952. https://doi.org/10.1038/nrmicro1553

Storteboom, H., Arabi, M., Davis, J. G., Crimi, B., & Pruden, A. (2010). Tracking antibiotic resistance genes in the South Platte River basin using molecular signatures of urban, agricultural, and pristine sources. *Environmental Science & Technology*, *44*, 7397–7404.

Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases*, *18*(5), 741–749. https://doi.org/10.3201/eid1805.111153

Thanner, S., Drissner, D., & Walsh, F. (2016). Antimicrobial resistance in agriculture. *MBio*, *7*(2), 1–7. https://doi.org/10.1128/mBio.02227-15

Verhougstraete, M. P., Martin, S. L., Kendall, A. D., Hyndman, D. W., & Rose, J. B. (2015). Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. *Proceedings of the National Academy of Sciences*, *112*(33), 10419–10424. https://doi.org/10.1073/pnas.1415836112

Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., Ii, R. P. M., & Ii, R. A. P. M. O. (2005). The urban stream syndrome: current knowledge and the search for a cure. *J. N. Am. Benthol. Soc.*, *24*(3), 706–723.

Washington, V. J., Lear, G., Neale, M. W., & Lewis, G. D. (2013). Environmental effects on biofilm bacterial communities: A comparison of natural and anthropogenic factors in New Zealand streams. *Freshwater Biology*, *58*(11), 2277–2286. https://doi.org/10.1111/fwb.12208

Watkinson, A. J., Micalizzi, G. B., Graham, G. M., Bates, J. B., & Costanzo, S. D. (2007). Antibiotic-resistant *Escherichia coli* in wastewaters, surface waters, and oysters from an urban riverine system. *Applied and Environmental Microbiology*, *73*(17), 5667–5670. https://doi.org/10.1128/AEM.00763-07

Wellington, E. M. H., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., & Williams, a P. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*, *13*(2), 155–165. https://doi.org/10.1016/S1473-3099(12)70317-1

Whitlock, J. E., Jones, D. T., & Harwood, V. J. (2002). Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research*, *36*, 4273–4282.

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag.

Winkworth, C. L. (2013). Antibiotic resistance genes in freshwater biofilms along a whole river. *Journal of Water and Health*, *11*(2), 186–198. https://doi.org/10.2166/wh.2013.223

Winter, C., Hein, T., Kavka, G., Mach, R. L., & Farnleitner, A. H. (2007). Longitudinal changes in the bacterial community composition of the Danube River: a whole-river approach. *Applied and Environmental Microbiology*, *73*(2), 421–431. https://doi.org/10.1128/AEM.01849-06

World Health Organization (WHO). (2014). *Antimicrobial Resistance: 2014 Global Report on Surveillance*.

Wu, J. (2004). Effects of changing scale on landscape pattern analysis: scaling relations. *Landscape Ecology*, *19*(2), 125–138. https://doi.org/10.1023/B:LAND.0000021711.40074.ae

Yang, H., Vinopal, R. T., Grasso, D., & Smets, B. F. (2004). High diversity among environmental *Escherichia coli* isolates from a bovine feedlot. *Applied and Environmental Microbiology*, *70*(3), 1528–1536. https://doi.org/10.1128/AEM.70.3.1528

Yang, X., Liu, Q., Luo, X., & Zheng, Z. (2017). Spatial regression and prediction of water quality in a watershed with complex pollution sources. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-08254-w

Zhou, Z., Zheng, J., Wei, Y., Chen, T., Dahlgren, R. A., Shang, X., & Chen, H. (2017). Antibiotic resistance genes in an urban river as impacted by bacterial community and physicochemical parameters. *Environmental Science and Pollution Research*, *24*, 23753–23762. https://doi.org/10.1007/s11356-017-0032-0

Chapter 3 : Secondary Productivity of *Daphnia magna* Raised with Novel Bacterial Symbionts

Angela M. Klock[1], Michael Brett[2]

[1] School of Environmental and Forest Sciences, University of Washington, Seattle WA, U.S.A. 98195

[2] Department of Civil and Environmental Engineering, University of Washington, Seattle WA, U.S.A. 98195

To be submitted to Environmental Science & Technology

Abstract

Urban aquatic systems are pervasively contaminated by anthropogenic sources of bacteria. These pollutants interfere with ecological interactions and disrupt food web bioenergetics. Symbiotic bacteria are critical components of zooplankton communities and modulate numerous physiological processes of their hosts. The goal of this study was to quantify the growth rate, productivity (fecundity) and nutritional value (fatty acid profile) of *Daphnia magna* grown with bacteria from distinct ecologies (clinical, symbiotic, and environmental). *Daphnia* are important prey species comprising primary consumers near the base of the aquatic food web. To gain insights into how bacteria commonly found in polluted urban surface waters influence productivity individual *Daphnia* neonates (< 12 h old) were raised with three different bacterial inoculants, two cultivated from a polluted lake in Seattle, WA, USA, and one laboratory strain of *Escherichia coli*. The total number of neonates during the studied period was significantly higher for females grown with an environmental bacterial epibiont. A heuristic linear method was used to calculate growth rates from the number of neonates produced. The maximum growth rate ($r = 0.599$) was for *Daphnia* grown with the wild epibiont. Productivity of primary consumers that has implications for energy transfer to higher trophic levels. These findings indicate that environmental pollution that includes determinants for antibiotic resistance has implications for food web bioenergetics.

Key words: Daphnia, epibionts, antibiotic resistance, fatty acids.

1. Introduction

Prokaryotic organisms are the most metabolically diverse life forms and drive the Earth's biogeochemical cycles (Falkowski, 2008). All eukaryotic organisms have bacterial symbionts that perform a variety of functions including participating in the exchange of energy, nutrients, and metabolites with their hosts. Symbionts may be vertically transmitted, usually through the female, and therefore heritable (Ferrari & Vavre, 2011), or acquired horizontally from the environment with each new generation (Bright & Bulgheresi, 2010). Bacterial symbionts foster the acquisition of nutrients that are limiting and thereby open niches to their insect hosts (Feldhaar, 2011), and directly or indirectly affect interactions between species (Ferrari & Vavre, 2011). Species interactions are key to understanding ecosystem-level processes and eukaryotes harbor complex communities comprising diverse microbes, some obligate, some facultative, specialists or generalists, representing a wide range of inter-specific symbiont-host interactions. For practical purposes we can consider a host and its vertically transmitted symbionts a single unit for natural selection, the 'holobiont' (Zilber-Rosenberg & Rosenberg, 2008) whereby the hosts' heritable genetic variation is increased. The genetic diversity of microbial symbionts may be critical for both adaptation and evolution of higher organisms particularly in the face of rapid climate change. The rapid generation time and capacity for horizontal gene transfer allows microbes to rapidly respond to changes in the environment. A microbiome that responds "on the fly" can provide the ecological cushion required by the holobiont to survive rapidly changing environments.

As eukaryotic microbial symbionts are key participates in the remineralization of organic material and the transformation of key elements, carbon, nitrogen, phosphorous, and sulfur between inorganic forms, they directly influence the flux and transformation of energy and matter into animal biomass (Beinart, 2019). In animals, the most diverse and complex metabolically microbiome is

found in the gut. The gut microbiome contributes to host adaptation as the collection of microbial genomes is more genetically diverse than the host genome, evolves faster, and can be rapidly altered by the environment (Shapira, 2016). It has been proposed that the importance of this function differs by habitat and some host-associated microbes may play an outsized role relative to their functionally equivalent free-living microbes (Beinart, 2019). *Daphnia* is a common, widespread keystone genus of more than 100 species of freshwater planktonic zooplankton in temperate and cold freshwater ecosystems (Ebert, 2005) and an important link between primary productivity and higher levels of the trophic pyramid (Ebert 2005; Lampert 2006). *Daphnia* have been extensively studied and is a widely used model organism in toxicology and aquatic ecology (Asaeda & Acharya, 2000). An analysis of the symbionts of laboratory clones of *D. magna* revealed diverse communities dominated by Proteobacteria, specifically, the Betaproteobacteria (~63%) and Gamaproteobacteria (~27%) where *Escherichia coli* (*E. coli*) was the most common taxa present in the class (Qi et al., 2009). In addition, Qi et al, found strong similarities at the genus and higher taxonomic level among three laboratory *Daphnia* species, but the three clones hosted different species. He concluded that similarities among the symbiont communities may be long-standing and stable in the genus *Daphnia*. The microbiome can be considered as a continuum of functions from a host-adapted core microbiome that is highly conserved and performs key metabolic functions such as transport of vitamins and amino acid biosynthesis (Cooper & Cressler, 2020). There are six amino acids demonstrated as likely essential for *Daphnia* spp. arginine, histidine, leucine, phenylalanine, isoleucine, and tryptophan. Each of these have shown to be biosynthesized by at least one common bacterial symbiont of *Daphnia* (Cooper & Cressler, 2020). The permanence of these types of host-symbiont relationships has implications for the success of *Daphnia* as a productive prey item in polluted surface waters.

At the other end of the spectrum, is a flexible, environmentally-sensitive microbiome that is in flux with the environment and contributes uniquely to host adaptative capacity based on site-levels conditions (Shapira, 2016). Microbial symbionts thereby play a critical ecological role as determinants of the productivity and food value of primary and secondary consumers near the base of the food web, and also as tools for host adaptation.

There is evidence of significant impacts to food webs initiated by temperature shifts (Shurin et al., 2012) and aquatic invertebrates are uniquely exposed to environmental bacteria that attach to their bodies or are ingested while feeding. In aquatic systems novel environmental bacteria may arise from the degradation of lakes and rivers by development which is an ongoing critical global issue due to its importance to human health. The pollution of urban lakes and rivers is well documented but there remain knowledge gaps as to how this impacts the food web. Freshwater ecosystems are potentially inundated by many different sources of pollutants including excess nutrients which causes eutrophication, and anthropic sources of bacteria. As nutrient composition and stoichiometry is regulated by bacterial biogeochemical processes these perturbations propagate up through the food web. Thus, the complex interaction of multiple feedbacks alters the nutritional status of primary consumer prey species as their symbiotic relationships are altered. Empirical evidence is needed to understand how bacterial community composition is determinative of ecological function at multiple levels of biological organization (G. Singer et al., 2010).

A familiar system-state change in urban surface waters is eutrophication, that results from excess nutrients (Paerl et al., 2001). In these highly productive systems, bacterial-mediated nutrient cycles respond to the composition and levels of nutrients and are determinative of system-state shifts (De Vries & Hopstaken, 1984). Cyanobacteria and other harmful algae are sensitive to excess phosphorus (Havens et al., 2003) as these organisms are adapted to be superior scavengers for

phosphorus where they will grow rapidly until nutrients are depleted and they die. Decomposition produces an increased biological oxygen demand stripping the water column of oxygen. The types of "blooms" are common in urban surface waters where *Daphnia* are important prey for small fish. Cyanobacteria are poor food for *Daphnia* and their populations decline and become less nutritious where these algae come to predominate (Brett et al., 2006). Lake Washington Seattle is probably the best example of a successful recovery of a large urban lake. Secondary treated sewage was released directly into Lake Washington from 1942 – 1963 and resulted in extensive eutrophication. As cyanobacteria overgrew the lake a tipping point was crossed, and the ecology of the lake was severely altered. After effluents were diverted years later the cyanobacteria died back and have been relatively insignificant since about 1976. The subsequent drop in measured phosphorus did not fully account for the rapid clearing of the lake and it was determined that feedbacks between a smelt, a shrimp, and *Daphnia* were responsible for the lake's relatively quick recovery and return to mesotrophic status. *Daphnia* productivity played a central role in this as a primary prey item for small fish and their nutritional value was due to the available high-quality phytoplankton and their bacterial symbionts. In moderately productive systems trophic interactions are more diverse and complex, and there is generally higher biodiversity (Jeppesen et al., 2000). These relationships have been described across many lake systems where ecosystem shifts occur relative to cyanobacterial abundance (Schindler, 2012).

Daphnia magna are parthenogenetic most of the year and mature females produce between 3-100 neonates every three to seven days depending on food quantity and quality and water temperature. *Daphnia* filter feed on a wide variety of phytoplankton, protists, and bacteria (Sherr & Sherr, 2002). The carbon acquired from food is then passed to their offspring (Hadas et al., 1983). Subsequently, the characteristic fatty acid profile of the food is represented in the fatty acid profile

of *Daphnia* which ultimately is passed up the aquatic food web (Brett et al., 2006). This results in a gradient of energy and nutrient transfer across multiple trophic levels (Breck, 2008). There are some studies that have looked specifically at how bacteria influence *Daphnia* productive performance (Eckert & Pernthaler, 2014). For example, growth as a function of bacterial food sources (Taipale et al., 2012), metabolic changes with food supplementation and exposure to pathogens (Schlotz et al., 2014), the effects of bacterial traits to symbionts and feeding behavior (Gorokhova et al., 2015), or the facilitation of the uptake of dissolved organic carbon by bacterial epibionts (Eckert & Pernthaler, 2014). If anthropic-based bacterial pollutants mediate the abundance and quality of *Daphnia* as a food source this has important consequences for trophic cascades in urban lakes and rivers. Systematic energy loss and lower nutritional value of primary consumers is particularly relevant in urban surface waters that function as rearing habitat for economically and ecologically important fish such as salmon. To protect these species, regulatory agencies need to understand the ecological dynamics of the food web in systems where disturbance, pollution and human-use coincide with critical wildlife habitat.

We want to explore how common environmental bacteria influence the growth and ecological fitness of a primary consumer important to higher trophic levels. Here we replicate environmental exposure to bacteria from a polluted lake and simulate the physiological processes that may occur when these types of bacteria interact with freshwater zooplankton throughout their life history. The goal of this experiment was to quantify the growth and reproduction of *Daphnia* when exposed to bacteria from different primary ecologies. The objectives were to test the following hypotheses:

1) Naïve *daphnids* confronted by novel bacterial strains will have different physiological responses due to a fitness cost or benefit imposed by the bacteria in their environment. These fitness impacts will be reflected as changes in growth and reproductive metrics.

2) *Daphnia* will metabolize different fatty acid profiles (nutritional value) in response to the presence of these bacteria due to changes in bioenergetics or other cumulative effects on physiology.

2. Methods

2.1. Model zooplankter *Daphnia magna*

This analysis was conducted using laboratory cultures of *Daphnia magna* (*D. magna)* originally isolated from Clear Lake, California. *Daphnia* were maintained with a constant temperature (20 ± 1 °C) and light : dark cycle (14 h : 10 h) in the Riverine Ecology laboratory at the School of Environmental and Forest Sciences, University of Washington Seattle (UW). *Daphnia* were fed a non-axenic culture of the green alga *Scenedesmus obliquus* cultivated in 2 L Erlenmeyer flasks under the same illumination light schedule in the Lake Research Laboratory at the School of Environmental and Civil Engineering, UW. The concentration of algae was determined with a Turner Designs 10-AU fluorometer calibrated with a known chlorophyll-α standard (Sigma) to determine the chlorophyll content of live cultures. *Scenedesmus* and *Daphnia* cultures were maintained on L16 growth media (Lindström, 1983).

One-day old *Daphnia* neonates (n = 30) were selected as "founders" and cultured at 20 °C in thirty separate 50 ml glass scintillation vials under a 14 : 10 dark-light cycle, with daily feeding *ad libitum* of fresh log-phase *S. obliquus*. Daphnids were observed daily, and the number of clutches noted. To control for maternal effects, neonates < 24 h old (n = 60) were taken from the 3rd and

4th clutches of the founders as replicates for the experimental treatments. Hatchlings were removed and washed with fresh L16 media to remove unattached bacteria that might serve as a food source for the *Daphnia*. An individual daphnid was placed in each of ten 50-mL scintillation vials per treatment (n = 5) on day one and monitored daily. All *Daphnia* were fed *S. obliquus* as above.

2.2. Novel bacterial treatments

To provide a baseline for regular laboratory growth metrics a "control" treatment of *Daphnia* was grown under normal laboratory conditions with the naturally occurring bacterial epibionts present on the mothers (i.e., composition and concentration of bacteria present from the media and grow conditions of laboratory maintenance). To test for differences of growth and fecundity due to common exogenous bacteria we confronted *Daphnia* with two bacterial treatments from a polluted environmental source and one laboratory strain of *E. coli*. To control for the effects of the bacterial growth medium we supplemented a fifth treatment with no bacterial additive 2.0 mL of Luria–Bertani medium (LB) (Sigma, St. Louis, MO, USA).

We collected our environmental samples after an extended period of dry weather at Sakuma Viewpoint, Lake Union Seattle (47.651557, -122.314872 [UTM 551450.55, 5277800.77]). An epilithic biofilm was collected from shoreline rip rap and an Ephemeroptera larva (*Hexagenia* spp.) was collected from the substrata at the same location. The biofilm was collected with the eSwab™ collection and preservation system (Fisher Scientific, City, State, USA) and the invertebrate was collected with a sterile sampling bottle. Samples were stored on ice and processed within 24 h.

In the laboratory an eSwab moistened with sterile water was rubbed over the dorsal surface of the mayfly thorax and abdomen to collect the bacterial epibionts. We repeated the swabbing procedure with a sample of ten adult *Daphnia* from the main culture. The eSwab samples containing

the epibionts and the biofilm were streaked onto fresh MacConkey agar plates and incubated at 37.5 °C for 24 h and observed for growth. The mayfly and biofilm plate contained vigorous growth of Gram-negative rods (GNR). There was no growth observed on MacConkey plates from the *Daphnia* swabs. We selected several isolates from the most common colony type present and replated for isolation. We then processed the samples through a series of Gram-staining and biochemical tests to determine the most probable identification of each isolate. We then identified the antibiotic resistance profile of each isolate.

The biofilm isolate was a presumptive enteric Gram-negative rod (eGRN) Gammaproteobacteria *E. coli* resistant to ampicillin and chloramphenicol. The epibiont was a presumptive non-enteric Gram-negative rod (neGRN) Betaproteobacteria of the genus *Limnohabitans* resistant to six antibiotics. All bacterial cultures were maintained in LB broth under refrigeration. Prior to inoculation into the *Daphnia* vials bacterial cultures were grown at room temperature in liquid media for 12 h to log phase. The cell concentration was then adjusted to OD_{600} 1.0 (~1.0 x10^8 CFU / mL) with a Genesys 10 UR Thermo Spectrophotometer (Themo Fisher Scientific Inc., Waltham, MA).

2.3. Bacterial antibiotic resistance traits

To determine the level of antibiotic resistance of the environmental samples we collected ten isolates from each sample (biofilm, epibiont) and inoculated into twenty-10 ml sterile glass vials (n = 10 each) containing 2 ml of non-selective Luria-Bertani medium (LB) and allowed to grow for 48 hours at room temperature. A swab from each vial was subsequently plated onto ten fresh MacConkey agar plates prepared with ampicillin (n = 20), chloramphenicol (n = 20), streptomycin (n = 20), spectomycin (n = 20), sulfamizole (n = 20), and tetracycline (n = 20). The plates were incubated at 36.5 °C for 24 h and examined for growth (n = 120). Growth indicated resistance to the

antibiotic at the threshold concentration. Through a series of isolation plating, we tested isolates for resistance to ampicillin (25 mcg / mL), chloramphenicol (30 mcg / mL), streptomycin (100 mcg / mL), spectinomycin (100 mcg / mL), sulfonamide (256 mcg / mL), and tetracycline (25 mcg / mL).

2.4. Experimental setup

Single daphnids were placed in a sterile 50 ml glass vial with fresh L16 medium and inoculated with 10 ul of LB broth or 10 ul the bacterial treatment inocula. If a neonate died within 24 h, it was replaced. Every three days the culture media was replaced, and fresh bacterial inoculum added. Vials were checked daily, and newly released neonates were removed to a new glass vial and maintained under the same conditions as the mothers. The date of the release and the count of neonates in each clutch recorded. A random sample of each clutch from each treatment were measured for length at the time of removal from the mother vial. All neonates were counted daily to measure survivorship. The experiment was conducted for 30 days. The reproductive performance metrics were fecundity (clutch size, cumulative production), age of first clutch release, and survival. The growth performance metric was length. The length of adults was taken at the end of the experiment and used to estimate "no observed bacterial effects." The intrinsic population growth rate (r) was calculated. Body length of mothers and offspring (91–260) individual measurements per treatment) were measured from the top of the eye to the base of the caudal spine to the nearest 0.02 mm and processed with image analysis (*sensu* Agatz et al., 2015). The five treatments were; i) "Control" no additive, ii) "Media" supplemented with 100 μL LB broth, iii) "Epibiont" 100 μL neGNR epibiont, iv) "Biofilm" 100 μL eGNR, and v) "ATCC" 100 μL *E. coli* (ATCC 25922).

2.5. Fatty acid quantification with gas chromatography - mass spectrometry

Daphnia - Fatty acid methyl esters from the *Daphnia* samples (Kattner & Fricke, 1986) were analyzed with a gas chromatograph (GC) (HP6890) equipped with a programmable temperature vaporizer injector, a fused silica capillary column (DB-23, J&W Scientific; 30 m length, 250 μm diameter, with 0.15-mm film thickness), and a flame ionization detector. A one-ml sample was injected with helium as the carrier gas. We used the following temperature program: 50 C° held for 1 min, then heated at 10 C°/min to 100 C°, held for 0 min, heated at 2 C°/min to 140 C°, held for 0 min, then 1 C°/min to 180 C°, held for 5 min, 2 C°/min to 200 C°, with no hold, then finally 10 C°/min to 240 C°. Individual FAs were identified based on the retention times of fatty acid methyl ester standards (Supelco 37 component FAME mix) dissolved in n-hexane and by comparison with retention times of other samples analyzed by Gas Chromatography - Mass Spectrometry (GCMS).

2.6. Statistical analysis

The growth rate of a population is a direct measure of fitness. We determined the maximum population growth rate from the log-linear part of a growth curve using a heuristic approach with the R package "`growthrates`" (Petzoldt, 2020) (Coors & Meester, 2008). The calculations applied through this function are based on Hall et al. (2013) and use linear regression to find the highest rate of exponential growth from a complete dataset or subsection. The effect of different treatments on growth, survival and fecundity of *Daphnia* were tested for normality of distribution (Multivariate Shapiro-Wilk normality test, $p > 0.05$). Data that did not satisfy assumptions of normal distribution or equal variances were analyzed with non-parametric Wilcoxon Signed Rank test for comparison of the treatments to the control before analysis of variance (ANOVA). *Post-hoc* comparisons were made by Dunnett's test.

Fatty acids were categorized into six groups: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), phospholipid-derived fatty acids (PLFA), polyunsaturated fatty acids (PUFA), highly unsaturated fatty acids (HUFA), and the ratio of w3 to w6 FAs (w3/w6 ratio). These six fatty acid categories were selected because they represent important fatty acid functional groups in zooplankton and represent the nutritional value of *Daphnia* to higher trophic level. This classification scheme also served to reduce the complexity of the individual fatty acids detected in the samples (Table 3-1).

Table 3-1: Nomenclature and structure of fatty acids tested.

Type	Structure	Abbreviation
Saturated fatty acids	C14:0, C15:0 (iso), C16:0, C18:0,	SAFA
Monosaturated fatty acids	16:1ω9, 18:1ω9, 18:1ω7	MUFA
Polyunsaturated fatty acids	14:1ω7, 16:1ω7, 16:2ω6, 16:3ω3, 16:4ω3, 18:2ω6, 18:3ω6, 18:3ω3, 18:4ω3	PUFA (EFAs)
Highly unsaturated fatty acids	20:4ω6, 20:5ω3	HUFA
Phospholipid derived fatty acids	15:1n9 (branched), C15:0 (anteiso), C17:0 (iso), 17:1ω7	PLFA
ω3 PUFA	ALA 18:3ω3; SDA 18:4ω3, EPA 20:5ω3	
ω6 PUFA	LIN 18:2ω6, GLA 18:3ω6, ARA 20:4ω6	
Bacterial fatty acids	C15:0 (iso), 15:1n9 (branched), C15:0 (iso-antiso), C17:0 (iso)	BAFA

Principle component analysis (PCA) was used to visualize the results of the fatty acid groups in semantic space. The fatty acid composition was analyzed with the R package "`FactoMineR`" (Le et al., 2008) and "`factoextra`" (Alboukadel Kassambara & Mundt, 2020). All statistical analysis was performed in R version 1.3.1093 (R Core Team, 2020).

3. Results

3.1. Neonate survivability

The proportion of surviving neonates from the first clutch were [Control > Biofilm > Media > Epibiont > ATCC]. This may indicate adaptation of the clones to the laboratory grow conditions as all treatments showed at least 30% higher mortality than the Control with the first clutch. Neonate survival improved with subsequent clutches as Media, Epibiont, and Biofilm treatments produced the highest total neonates at the end of the experiment. This may indicate that the bacterial treatments provided additional nutritional value or some other physiological benefit. Although the age of primiparous daphnids differed by up to 4.3 days between treatments, the length of the first clutch offspring were invariant to adult length and neonate survival (Table 3-2).

At 30 days the total number of neonates released differed by a maximum of 58% based on treatment. This was due in part to early maternal mortality. All treatments suffered female daphnid losses although ATCC had 90% female mortality. Despite this ATCC produced slightly more neonates then Control.

Table 3-2: Summary of experimental demographics for *Daphnia magna* treatments.

Treatment	Maternal age primiparous clutch mean ± SEM	Range of age primiparous clutch (days)	Size of primiparous clutch (ind^{-1}) mean ± SEM	Total neonates
Control	9.6 ± 0.44	8-11	6.7 ± 0.63	139
Media	10.6 ± 0.31	10-12	11.8 ± 0.76	232
Epibiont	9.9 ± 0.82	8-17	7.0 ± 1.19	238
Biofilm	12.1 ± 0.78	9-17	7.6 ± 1.25	199
ATCC	13.9 ± 1.31	9-19	8.2 ± 1.22	141

Treatment	Maternal length (mm) mean ± SEM	Length of primiparous clutch (mm) mean ± SEM	Neonate survival %
Control	4.12 ± 0.06	2.64 ± 0.05	48.2
Media	4.06 ± 0.20	2.29 ± 0.05	65.1
Epibiont	4.19 ± 0.05	2.54 ± 0.08	76.5
Biofilm	3.95 ± 0.19	2.11 ± 0.07	82.9
ATCC	4.40 ± 0.07	2.52 ± 0.07	68.1

3.2. Population growth rate

Population growth accounted for mortality of adults and neonates and was independent of maternal age and clutch size at primiparous release, i.e., the Epibiont treatment produced the largest number of neonates overall and had the fastest growth rate but matured later than Control and had the second smallest primiparous clutch size. The highest growth rates were [Epibiont > Biofilm > ATCC > Control > Media]. The better performance of Control over Media may be due to the slowed growth in Media after day 15. The only other treatment that duplicated this late-stage growth was ATCC performing just higher than the Control after day 14. The two highest slopes originated before day 10 (Table 3-3, Fig. 3-1).

Table 3-3: Maximum growth rates from the log-linear part of a growth curve using the "growthrates" algorithm regression analysis. R-squared is how close the data are to the fitted regression line and deviance is the residual sum of squares.

Treatment	*F*	*p*-value	Maximum growth rate (slope)	R^2	Deviance (residual SS)
Control	9.452	0.054	0.208	0.76	0.293
Media	11.7	0.042	0.125	0.80	0.136
Epibiont	54.05	0.005	0.599	0.85	0.199
Biofilm	173.9	0.000	0.479	0.98	0.039
ATCC	54.37	0.005	0.245	0.95	0.033

Consistent with the major differences in fecundity for *Daphnia* supplemented with growth media and bacteria, *Daphnia* supplemented with Media and environmental bacteria grew at very high rates that was not reflected by the size of their first clutches, whereas *Daphnia* supplemented with ATCC took longer to mature and had poor fecundity. In contrast, the nutritional benefits of Media jump-started production with high primiparous clutch size but the level of production was not sustained and declined to less than five neonates per female in subsequent clutches.

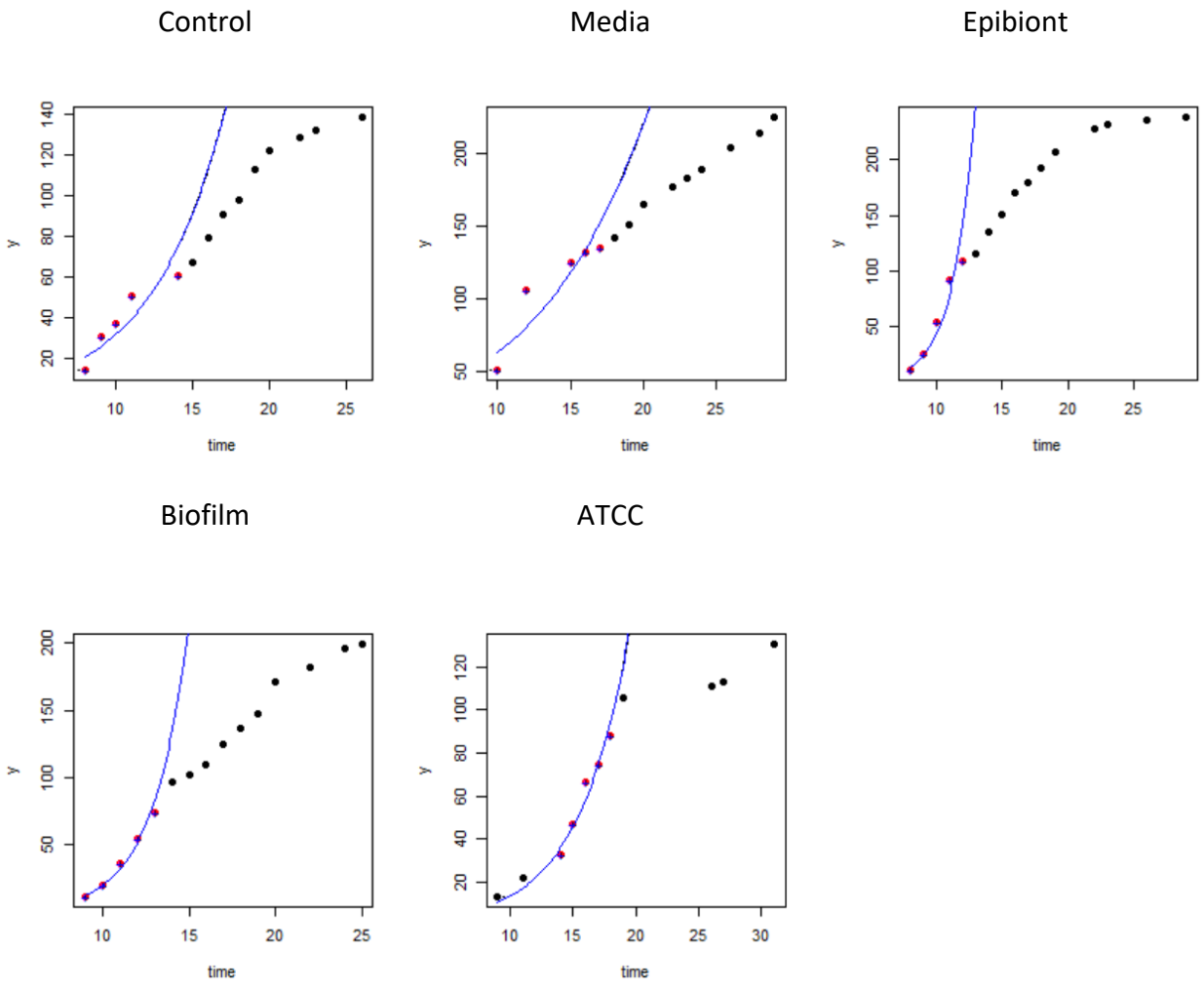


Figure 3-1: Maximum growth rates of *Daphnia magna* populations from the log-linear part of a growth curve using a heuristic approach. The red dots represent the number of data points (5) that are within 95% of the maximum slope. Treatments (A) Control, (B) Media, (C) Epibiont, (D) Biofilm, and (E) ATCC.

3.3. Productivity metrics

A series of one-way analysis of variance (ANOVA) to test six reproductive traits indicated significant effects of treatment for neonates per female, age at primiparous release, length of primiparous neonates, total fecundity, and neonate survival. Maternal size at 30 days were similar among all treatments ($p > 0.05$).

Total fecundity was increased in all treatments over the Control (Epibiont > Media > Biofilm > ATCC > Control, $p = 0.031$). Post-hoc Dunnett's test indicated significantly higher neonates per female for treatments with environmental bacteria, where Epibiont increased fecundity 1.7x ($p = 0.04$, 95% CI (0.05, 3.98) and Biofilm increased fecundity 1.4x ($p = 0.02$, 95% CI (0.35, 4.41) (Table 3-4, Fig. 3-2).

Table 3-4: One-way analysis of variance (ANOVA) summary.

Source	SS	*df*	*F*	*p*	SS	*df*	*F*	***p***
	Maternal age primiparous clutch				Adult length			
Treatment	118	4	4.85	*0.003*	0.92	4	1.55	0.206
Error	261.31	43			5.37	36		
	Primiparous clutch size				Primiparous neonate length			
Treatment	175.22	4	4.11	*0.007*	7.13	4	8.01	*0.000*
Error	457.78	43			59.85	269		
	Fecundity (total neonates)				Proportion neonates survived			
Treatment	101.27	4	3.45	*0.031*	218.75	4	8.14	*0.000*
Error	1910.78	166			1115.78	166		

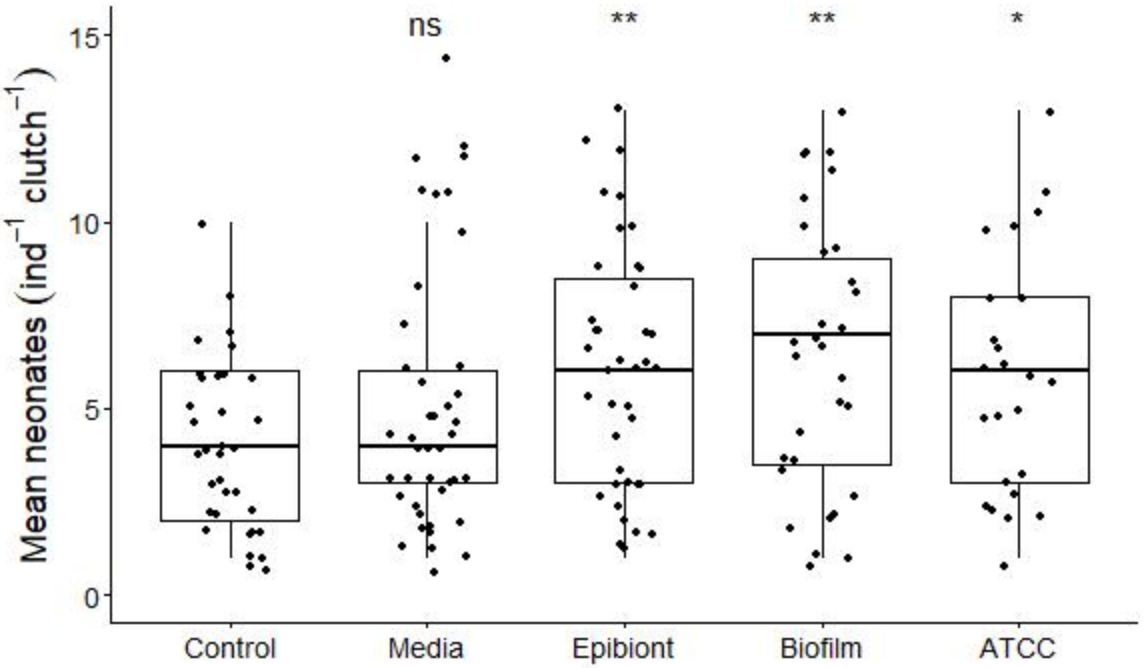


Figure 3-2: Mean neonates per female by treatment. ANOVA p-value indicated at least aone group was significantly different from the grand mean (m = 5.5) represented by the dashed line. Significant values above treatments indicate level of significant difference from the Control. As Media (W = 0.856, p < 0.000, Shapiro-Wilk normality test) did not conform to the assumption of normality, a Wilcoxon Signed Rank test was used for comparison of treatments to Control. Convention for symbols indicating statistical significance (ns; p > 0.05, *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001, ****: p <= 0.0001)

Maternal age at primiparous release was increased by treatment (Control < Epibiont < Media < Biofilm< ATCC, p = 0.003). The time to maturity increased by 2.6 days for Biofilm treatment (p = 0.09, 95% CI (-0.32, 5.41, Dunnett's test) and 4.3 days by ATCC treatment (p = 0.002, 95% CI (1.39, 7.27, Dunnett's test) (Fig. 3-3).

Bacterial treatment has a significant effect (p = 0.007) on the size of the primiparous clutch neonates at 30-days [Control < Epibiont < Biofilm < ATCC < Media] (Table 3-4). There were on average 5.4 additional neonates produced per female daphnid by the supplement of LB broth (p = 0.003, 95% CI (1.56, 9.15, Dunnett's test).

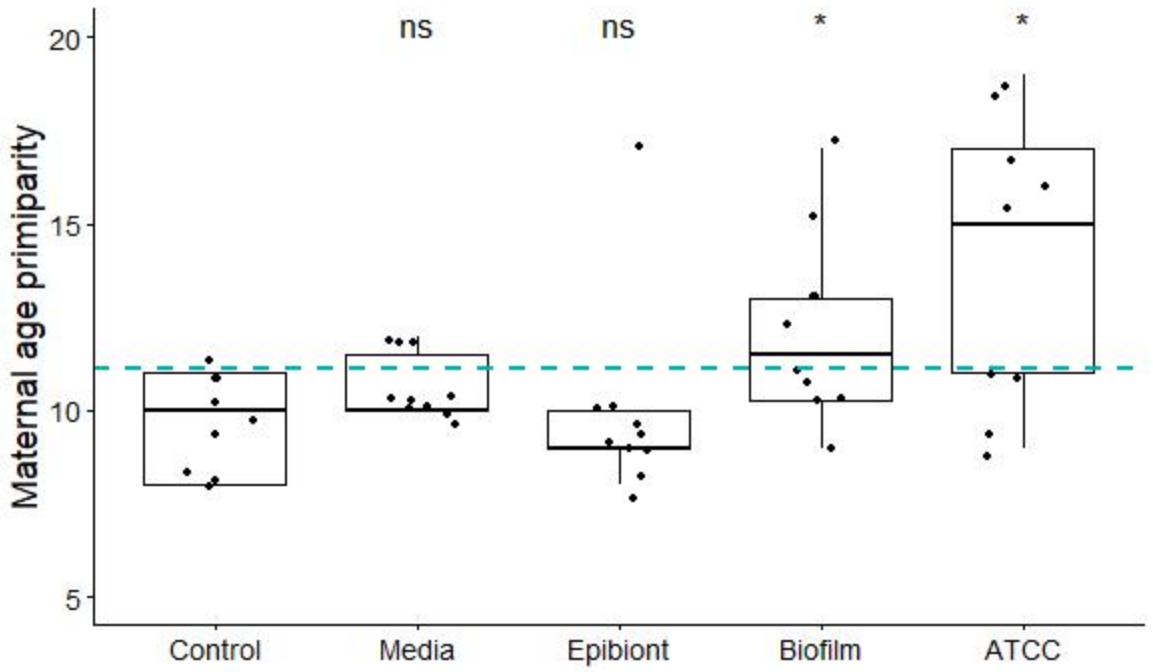


Figure 3-3: Mean maternal age at primiparous clutch release by treatment. ANOVA (p = 0.003) indicates at least one group is significantly different. Grand mean (m = 11.12) represented by the dashed line. Significant values above treatments indicate level of significant difference from the Control. As treatments Control ($W = 0.826$, $p < 0.04$), Media ($W = 0.594$, $p < 0.000$) and Epibiont ($W = 0.622$, $p < 0.001$) did not conform to the assumption of normality by Shapiro-Wilk normality test, a Wilcoxon Signed Rank test was used for comparison of treatments to Control. Convention for symbols indicating statistical significance (ns; p > 0.05, *: $p <= 0.05$, **: $p <= 0.01$, ***: $p <= 0.001$, ****: $p <= 0.0001$).

Total neonate survival over the 30-day experiment was over 69% in all treatments to the Control (Table 4). Dunnett's test revealed that survivability increased between 46% and 57% with the addition of Media ($p < 0.000$), ATCC ($p < 0.000$), Epibiont ($p < 0.000$) and Biofilm ($p < 0.000$). The Biofilm treatment increased survival by 2.3 times and the Epibiont treatment increased survival odds by 2.2 times.

Daphnia primiparous neonate length measured at experiment end was biased in some treatments due to delays in maternal maturity but was informative about the ability of neonates to recover growth over time (Table 3-4). A post hoc Dunnett's test revealed that neonate length averaged 0.35 mm less in Media ($p < 0.001$) and 0.53 mm less in Biofilm ($p < 0.001$) to Control. Accounting for the day of their release Media neonates grew 0.01 mm/day less and Biofilm neonates grew 0.03 mm/day less than Control (Fig. 3-4). The final length of adult lengths at the end of 30-days were similar.

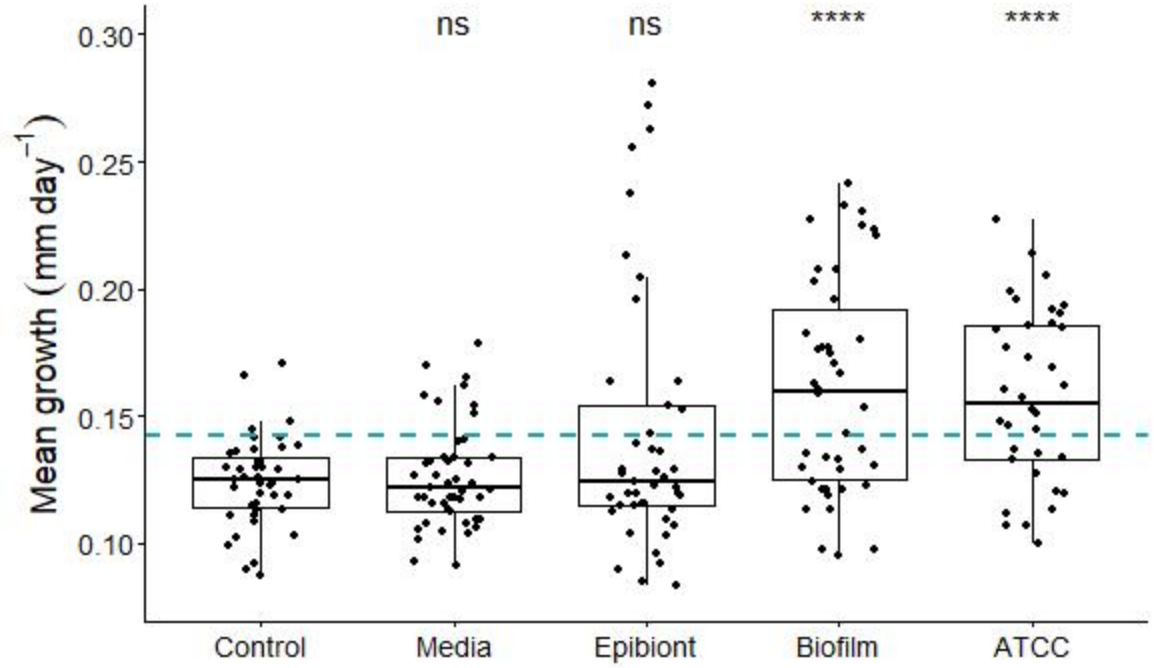


Figure 3-4: Mean growth rate of primiparous clutch. ANOVA ($p < 0.000$) indicated at least one group is significantly different. Grand mean (m = 0.142) represented by the dashed line. Significant values above treatments indicate level of significant difference from the Control. As treatments Media ($W = 0.943$, $p = 0.027$), Epibiont ($W = 0.811$, $p < 0.000$) and Biofilm (W=0.940, $p < 0.029$) did not conform to the assumption of normality by Shapiro-Wilk normality test, a Wilcoxon Signed Rank test was used for comparison of treatments to Control. Convention for symbols indicating statistical significance (ns; $p > 0.05$, *: $p <= 0.05$, **: $p <= 0.01$, ***: $p <= 0.001$, ****: $p <= 0.0001$).

The number of neonates produce per female decreased after clutch one in all treatments but remained relatively stable through clutch five among the treatments containing bacteria. Epibiont females were the most consistent, producing substantial numbers of neonates in each of five clutches in 30 days. Biofilm *Daphnia* remained highly productive through clutch three and then fell off rapidly producing one additional clutch with three females averaging less than two neonates each. The ATCC treatment also declined rapidly after the third clutch, with three females releasing a fourth clutch and a single female releasing a fifth clutch of 5 neonates.

3.4. Fatty acid profiles of *Daphnia* from each treatment

The green alga *S. obliquus* is medium quality food that contains sterols but is deficient in C20 PUFAs (Freese & Martin-Creuzburg, 2013). Comparisons of the *Daphnia* fatty acid composition (Table 3-5) show that *Daphnia* raised with Media and enteric bacteria (Biofilm, ATCC) had less saturated FAs and enteric bacterial treatments had less monounsaturated FAs than the non-enteric epibiont bacteria. Wild type *E. coli* from freshwater environments has been shown to contain the SAFA C19:0 and we identified C19:0 and C17:0 fractions in all treatments. Typically, PUFAs and sterols are not detected in common freshwater bacteria and *S. obliquus* contains no PUFAs with more than 18 carbon atoms but does contain high amounts of 18:2ω6 and 18:3ω3 (Freese & Martin-Creuzburg, 2013).

Table 3-5: Proportion of fatty acid group per treatment.

Treatment	∑ FA	SAFA	MUFA	PUFA	PLFA	HUFA	ω3/ω6
Control	1631.0	21.8	35.3	37.8	1.4	3.7	0.74
Media	1543.6	21.0	35.6	38.4	1.4	3.5	0.71
Epibiont	1434.6	23.0	33.1	38.4	1.8	3.8	0.75
Biofilm	2969.0	20.2	31.2	43.4	2.6	2.5	0.44
ATCC	1746.3	21.8	32.3	38.8	1.8	5.4	0.70

The results of the PCA separated the fatty acid groups and treatments. The first PC (PC1) explained 72.8% of the variability and was positively correlated with PUFA ($r = 0.48$) as well as the sum of PLFA ($r = 0.46$), and negatively correlated with the ratio of w3/w6 ratio ($r = 0.48$). The second PC (PC2) explained 19.3% of the variability and was positively correlated with HUFA ($r = 0.58$), and negatively correlated with MUFA ($r = -0.65$). The third PC (PC3) explained an additional 7.9% of variability and was positively correlated with SAF ($r = 0.70$) and HUFA ($r = 0.68$) and negatively correlated with SAFA ($r = -0.70$). PC vectors for proportions of SAFA, MUFA, PUFA, PLFA, HUFA, and the ratio of w3/w6 fatty acids describe variation and account for the varied influences of the treatments. PLFA, PUFA and w3/w6 ratio moderately influenced PC1, whereas MUFA, and HUFA strongly influenced PC2 (Table 3-6, Fig. 3-5).

Table 3-6: Correlation coefficients (*r*) between the three principle components and the six fatty acid groups used to generate them.

	PC1	PC2	PC3
SAFA	-0.357	0.423	-0.702
MUFA	-0.343	-0.647	0.065
PUFA	0.478	0.026	-0.012
PLFA	0.457	0.254	-0.174
HUFA	-0.302	0.578	0.675
ω3/ ω6 ratio	-0.476	0.053	-0.126

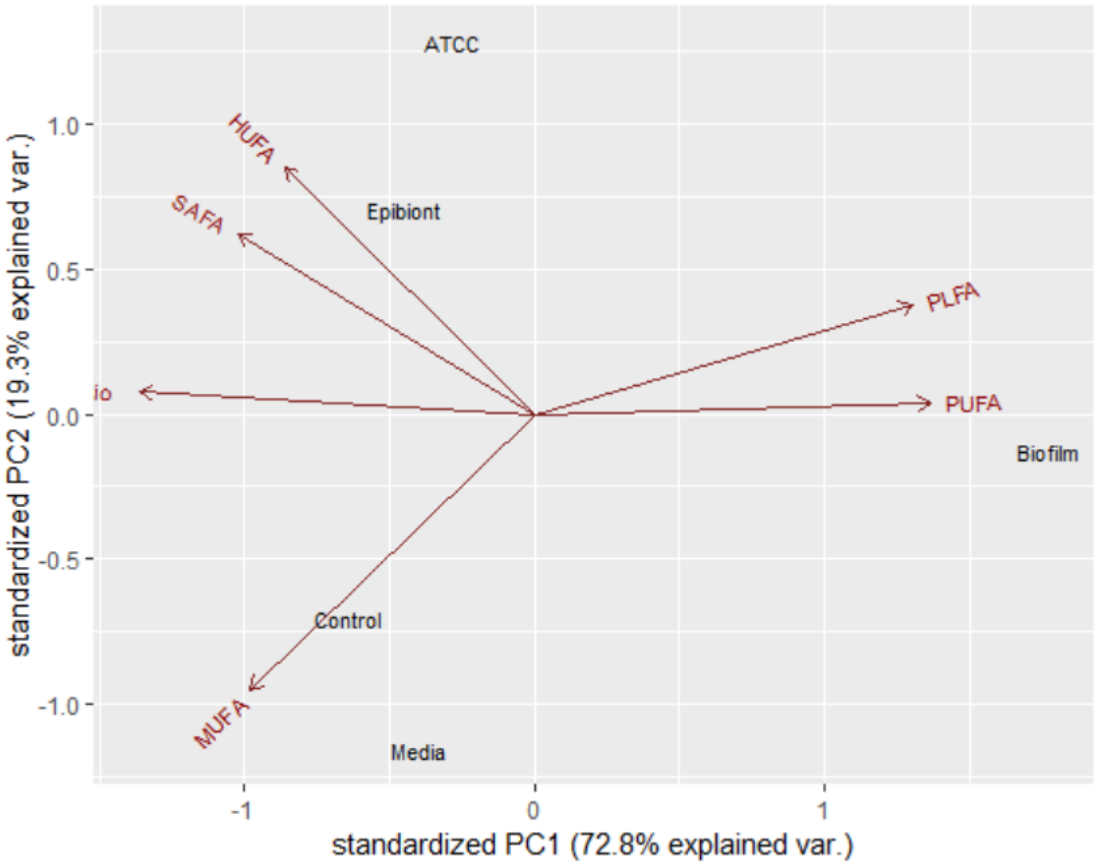


Figure 3-5: Biplot of principal components analysis (PCA) of fatty acid composition for the treatments and *Daphnia* samples. The first principal component (PC1) explained 72.8% of the overall variability and was positively correlated with PUFA (r = 0.48) as well as the sum of PLFA (r = 0.46), and negatively correlated with the ratio of w3/w6 ratio (r = 0.48). The second PC (PC2) explained 19.3% of the variability and was positively correlated with HUFA (r = 0.58), and negatively correlated with MUFA (r = -0.65). The third PC (PC3) explained an additional 7.9% of variability and was positively correlated with HUFA (r = 0.68) and negatively correlated with SAFA (r = -0.70). For example, Epibiont represents the FA composition of *Daphnia* with the treatment of the neGNR swabbed from the wild macroinvertebrate. PC vectors for proportions of SAFA, MUFA, PUFA, PLFA, HUFA, and the ratio of w3/w6 fatty acids describe variation and account for the varied influences of the original characteristics. Such influences (loadings) can be traced back from the PCA plot to find out what produces the differences among clusters. Vectors are pinned at the origin of PCs (PC1 = 0 and PC2 = 0) and their project values on each PC show how much weight they have on that PC. Here PLFA, PUFA and w3/w6 ratio strongly influence PC1, whereas MUFA, and HUFA influence PC2. The angles between the vectors tell us about the correlation between them. When two vectors are close, forming a small angle, the two variables they represent are positively correlated. If they meet each other at 90˚, HUFA and MUFA they are not likely to be correlated. When they diverge and form a large angle (close to 180˚), they are negative correlated, i.e., MUFA and PLFA or PUFA.

4. Discussion

The genus *Daphnia* comprises over a hundred species of Cladocerans that frequently dominate the zooplankton community in standing freshwater systems (Ebert, 2005). These organisms are critical to the flux of nutrients and energy between primary producers and secondary consumers (Peters & de Bernardi, 1987). *Daphnia* are non-selective filter-feeders so ingest a variety of phytoplankton and other microorganisms suspended in the water column. Heterotrophic bacteria frequently comprise a substantial portion of the suspended flocs where they play a critical role in the carbon cycle in the aquatic food web (Biddanda et al., 2001). Typically, algae comprise the bulk of the *Daphnia* diet and bacteria, although providing some nutrients, are primarily incidental (Urabe & Watanabe, 1991). Bacteria are poor sources of nutrition for daphnids as they do not contain sterols or PUFAs so do not contribute to somatic growth or reproduction (Martin-Creuzburg et al., 2011; Taipale et al., 2012). Seasonal changes and trophic status of aquatic systems determine the relative abundance of bacteria (Freese & Martin-Creuzburg, 2013). In our study, *Daphnia* were fed *ad libitum S. obligans*, an alga that has sustained growth and reproduction in laboratory clones for decades with the regular symbionts associated with laboratory cultures. Nevertheless, typical laboratory cultivation usually includes changing out the glassware and grow media weekly which would remove the biofilm on the glass and bacteria in the water. In our experiment, the neonates were started with the bacterial symbionts present on 6-hour old neonates. A small initial bacterial load may account for the low productivity of the Control treatment. Our results demonstrate that when a diet of algae is enhanced with bacterial growth medium and bacteria, all metrics of *Daphnia* productivity is enhanced. Bacteria may themselves serve as a nutrient or improve the digestion of food, provide additional vitamins or modulate other physiological processes.

Daphnia are not known to transmit bacterial symbionts inside their eggs but can be inoculated from bacteria attached to the surfaces of eggs or acquired from the environment after hatching (Sison-Mangus et al., 2015). *Daphnia* raised without bacteria produce fewer eggs and have lower survivorship compared to those raised with bacteria, and further neonates exposed even briefly to a single bacterial type from a non-*Daphnia* source grow as large as those raised continuously with bacteria (Sison-Mangus et al., 2015).

Daphnia require essential polyunsaturated fatty acids (PUFAs) and sterols in their diet (Brett & Muller-Navarra, 1997) which are frequently limited in eutrophic systems. To assess the effect of heterotrophic bacteria from different primary ecologies, environmental (macroinvertebrate epibiont, biofilm), and one laboratory strain of *E. coli* (ATCC 29522), on growth, survival, and fatty acid profile of *Daphnia* five treatments of bacteria–*Scenedesmus obliquus* mixtures were provided to day-old neonates in 50-mL microcosms. Diets that include heterotrophic bacteria have been found to significantly increase *Daphnia* growth rates compared to a pure algal diet (Freese & Martin-Creuzburg, 2013). We found that the addition of bacteria with the algae *S. obligans* nutritionally upgraded the diet and resulted in higher *Daphnia* fitness. Our results suggest that the phylogenetic composition and primary ecology of bacteria strongly influence *Daphnia* growth and reproduction and could potentially have similar influence on wild filter-feeding zooplankters.

Daphnia neonates provided with environmental bacteria were more than twice as likely to survive 30 days than neonates without added bacteria. The improved survival of *Daphnia* with the medium supplement may have been due to direct benefits of ingesting the nutrients in the broth or the growth and function of their natural symbionts was enhanced sufficiently to improve survivability and productivity of the *Daphnia* indirectly. The former seems more probable as the number of offspring in the first cohort was almost twice as large as any other treatment, but this

level of productivity did not recur with later clutches. The Media treatment did outperform the control by producing a fifth clutch as well which indicates better maternal condition over time.

A significant number of replicates with laboratory *E. coli* died after 6–12 days and produced no viable offspring, whereas those provided with phytoplankton diets enhanced with environmental bacteria had high survival, growth, and reproduction success. All *Daphnia* fed mixed bacteria and phytoplankton diets had evidence of bacteria specific FAs.

We demonstrate that *D. magna* laboratory clones responds uniquely to different bacterial treatments where the primary ecology of the symbiont appears to control *Daphnia* productivity. Our clones grew more slowly, produced fewer offspring and experienced higher mortality when raised in bacteria-impoverished environments or with the clinical strain of *E. coli*. We conducted our experiments with *D. magna* raised from lab-adapted parthenogenetic clones which controlled the natural variability of the host. Because *D. magna* needs to reach a minimum size to mature, adaptation to novel *E. coli* (Biofilm, ATCC) may explain the slow growth that resulted in the observed delay in maturity and neonate release with those treatments. This differed from the response to the wild macroinvertebrate epibiont, which resulted in rapid maturity and similar fecundity at primiparity. Although the range of days to primiparity for Control and Media was about three days, there was wide variability in the novel bacterial treatments, i.e., growing with the Biofilm *E. coli* an 8-day range, and Epibiont and ATCC with a 9- and 10-day range, respectively. This may indicate reproductive plasticity in response to ecological cues from different bacteria that selectively influence metabolism or other physiological responses of *Daphnia*. In this instance the proposed causal relationships suggest that exposure to novel bacteria increases fecundity among laboratory-reared clones, but secondary productivity is tightly coupled and specific to differences in the food value, symbiotic interactions, and/or ecology of the bacterial symbionts and the host. It is therefore reasonable to expect improved reproduction with the additional of a new food source, but this did

not hold for every bacteria type. The clinical strain resulted in high mortality and low fecundity. What needs to be assessed is how wild and sexually reproducing daphnids from pristine habitats would respond to similar bacterial treatments.

Acknowledgements

I want to extend my sincerest gratitude to Professor Michael Brett for his guidance and insights into the experimental procedure and results. This work could not have been possible without the equipment, chemicals, grow chambers and cultures from the Lake & Reservoir Laboratory at the Department of Civil & Environmental Engineering at the University of Washington. Special thanks to the technical expertise of Songlin Wang, and Lab & Instrumentation Manager, J. Sean Yeung for their thoughtful assistance in the processing of samples, providing standards, and helping with the use and interpretation of the GCMS.

References

Agatz, A., Hammers-Wirtz, M., Gergs, A., Mayer, T., & Preuss, T. G. (2015). Family-portraits for daphnids: scanning living individuals and populations to measure body length. *Ecotoxicology*, *24*(6), 1385–1394. https://doi.org/10.1007/s10646-015-1490-0

Alboukadel Kassambara, & Mundt, F. (2020). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses* (R package version 1.0.7). https://cran.r-project.org/package=factoextra

Asaeda, T., & Acharya, K. (2000). Application of individual growth and population models of *Daphnia pulex* to *Daphnia magna, Daphnia galeata* and *Bosmina longirostris*. *Hydrobiologia*, *421*(1–3), 141–155. https://doi.org/10.1023/A:1003955308697

Beinart, R. A. (2019). The Significance of Microbial Symbionts in Ecosystem Processes. *MSystems*, *4*(3), 1–5. https://doi.org/10.1128/msystems.00127-19

Biddanda, B., Ogdahl, M., & Cotner, J. (2001). Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnology and Oceanography*, *46*(3), 730–739. https://doi.org/10.4319/lo.2001.46.3.0730

Breck, J. E. (2008). Enhancing Bioenergetics Models to Account for Dynamic Changes in Fish Body Composition and Energy Density. *Transactions of the American Fisheries Society*, *137*(1), 340–356. https://doi.org/10.1577/T05- Beinart, R. A. (2019). The Significance of Microbial Symbionts in Ecosystem Processes. *MSystems*, *4*(3), 1–5. https://doi.org/10.1128/msystems.00127-19240.1

Brett, M. T., & Muller-Navarra, D. C. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, *38*, 483–499.

Brett, M. T., Müller-Navarra, D. C., Ballantyne, A. P., Ravet, J. L., & Goldman, C. R. (2006). *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography*, *51*(5), 2428–2437. https://doi.org/10.4319/lo.2006.51.5.2428

Bright, M., & Bulgheresi, S. (2010). A complex journey: transmission of microbial symbionts. *Nature Reviews. Microbiology*, *8*(3), 218–230. https://doi.org/10.1038/nrmicro2262

Cooper, R. O., & Cressler, C. E. (2020). Characterization of key bacterial species in the *Daphnia magna* microbiota using shotgun metagenomics. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-019-57367-x

Coors, A., & Meester, L. De. (2008). Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in *Daphnia magna*. *Journal of Applied Ecology*, *45*(NOVEMBER), 1820–1828. https://doi.org/10.1111/j.1365-2664.2007.0

De Vries, I., & Hopstaken, C. F. (1984). Nutrient cycling and ecosystem behaviour in a salt-water lake. *Netherlands Journal of Sea Research*, *18*(3/4), 221–245.

Ebert, D. (2005). *Ecology, Epidemiology and Evolution of Parasitism in Daphnia*. National Library of Medicine (US), National Center for Biotechnology Information. https://doi.org/10.1108/02634501111102760

Eckert, E. M., & Pernthaler, J. (2014). Bacterial epibionts of *Daphnia*: A potential route for the transfer of dissolved organic carbon in freshwater food webs. *ISME Journal*, *8*(9), 1808–1819. https://doi.org/10.1038/ismej.2014.39

Falkowski, G. (2008). *The Microbial Engines That Drive Earth's Biogeochemical Cycles*. *320*(May 2014), 1034–1039.

Feldhaar, H. (2011). Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology*, *36*(5), 533–543. https://doi.org/10.1111/j.1365-2311.2011.01318.x

Ferrari, J., & Vavre, F. (2011). Bacterial symbionts in insects or the story of communities affecting communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1569), 1389–1400. https://doi.org/10.1098/rstb.2010.0226

Freese, H. M., & Martin-Creuzburg, D. (2013). Food quality of mixed bacteria – algae diets for *Daphnia magna*. *Hydrobiologia*, *715*, 63–76. https://doi.org/10.1007/s10750-012-1375-7

Gorokhova, E., Rivetti, C., Furuhagen, S., Edlund, A., Ek, K., & Breitholtz, M. (2015). Bacteria-mediated effects of antibiotics on *Daphnia* nutrition. *Environmental Science and Technology*, *49*, 5779–5787. https://doi.org/10.1021/acs.est.5b00833

Hadas, B., Kott, Y., & Cavari, B. Z. (1983). Assimilation of *E. coli* cells by *Daphnia magna* on the whole organism level. *Hydrobiologia*, *102*(3), 163–169.

Hall, B. G., Acar, H. & Barlow, M. (2013). Growth Rates Made Easy. *Mol. Biol. Evol. 31*, 232-238, doi: 10.1093/molbev/mst197

Havens, K. E., James, R. T., East, T. L., & Smith, V. H. (2003). N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environmental Pollution*, *122*, 379–390. http://www.ncbi.nlm.nih.gov/pubmed/12547527

Jeppesen, E., Jensen, J. P., Sondergaard, M., Lauridsen, T., & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in Danish lakes: Changes along a phosphorus gradient. *Freshwater Biology*, *45*(2), 201–218. https://doi.org/10.1046/j.1365-2427.2000.00675.x

Kattner, G., & Fricke, H. S. G. (1986). Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *Journal of Chromatography*, *361*, 263–268.

Karako-Lampert, S., Hershkovits, G., Stambler, N., Simon-Blecher, N., Achituv, Y., Dubinsky, Z., & Katcoff, D.J. (2006). Differential gene expression in *Symbiodinium microadriaticum* clade B following stress. *Mar Biotechnol*, *8*(3):268-74. doi: 10.1007/s10126-005-5008-2.

Le, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Lindström, K. (1983). Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. *Hydrobiologia*, *101*(1–2), 35–47. https://doi.org/10.1007/BF00008655

Martin-Creuzburg, D., Beck, B., & Freese, H. M. (2011). Food quality of heterotrophic bacteria for *Daphnia magna*: Evidence for a limitation by sterols. *FEMS Microbiology Ecology*, *76*(3), 592–601. https://doi.org/10.1111/j.1574-6941.2011.01076.x

Paerl, H. W., Fulton, R. S., Moisander, P. H., & Dyble, J. (2001). Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *TheScientificWorld*, *1*, 76–113. https://doi.org/10.1100/tsw.2001.16

Peters, R.H., De Bernardi, R., (1987). *Daphnia. Memorie Dell 'Istituto Italiano di Idrobiologia*. p. 502.

Petzoldt, T. (2020). *growthrates: Estimate Growth Rates from Experimental Data* (R package version 0.8.2.). https://cran.r-project.org/package=growthrates

Qi, W., Nong, G., Preston, J. F., Ben-Ami, F., & Ebert, D. (2009). Comparative metagenomics of *Daphnia* symbionts. *BMC Genomics*, *10*, 172. https://doi.org/10.1186/1471-2164-10-172

R Core Team. (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. https://www.r-project.org/.

Schindler, D. W. (2012). The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society B: Biological Sciences*, *279*(August), 4322–4333. https://doi.org/10.1098/rspb.2012.1032

Schlotz, N., Pester, M., Freese, H. M., & Martin-Creuzburg, D. (2014). A dietary polyunsaturated fatty acid improves consumer performance during challenge with an opportunistic bacterial pathogen. *FEMS Microbiology Ecology*, *90*(2), 467–477. https://doi.org/10.1111/1574-6941.12407

Shapira, M. (2016). Gut Microbiotas and Host Evolution: Scaling Up Symbiosis. *Trends in Ecology and Evolution*, *31*(7), 539–549. https://doi.org/10.1016/j.tree.2016.03.006

Sherr, E. B., & Sherr, B. F. (2002). Significance of predation by protsts. *Antonie van Leeuwenhoek*, *81*, 293–308.

Shurin, J. B., Clasen, J. L., Greig, H. S., Kratina, P., & Thompson, P. L. (2012). Warming shifts top-down and bottom-up control of pond food web structure and function. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1605), 3008–3017. https://doi.org/10.1098/rstb.2012.0243

Singer, G., Besemer, K., Schmitt-Kopplin, P., Hödl, I., & Battin, T. J. (2010). Physical heterogeneity increases biofilm resource use and its molecular diversity in stream mesocosms. *PloS One*, *5*(4), 1–11. https://doi.org/10.1371/journal.pone.0009988

Sison-Mangus, M. P., Mushegian, A. a, & Ebert, D. (2015). Water fleas require microbiota for survival, growth and reproduction. *The ISME Journal*, *9*(1), 59–67. https://doi.org/10.1038/ismej.2014.116

Taipale, S. J., Brett, M. T., Pulkkinen, K., & Kainz, M. J. (2012). The influence of bacteria-dominated diets on *Daphnia magna* somatic growth, reproduction, and lipid composition. *FEMS Microbiology Ecology*, *82*(1), 50–62. https://doi.org/10.1111/j.1574-6941.2012.01406.x

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, *32*(5), 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

Chapter 4 : See the Forest not the Trees! Ecosystem-based Assessment of Response, Resilience, and Scope-For-Growth of Global Forests

Angela M. Klock, Kristiina A. Vogt, Daniel J. Vogt, John G. Gordon

To be submitted to Total Science of the Environment

Abstract

To assess the impact of climate change on the growth of vegetative communities at the local or ecosystem scale needs an ability to identify the "safe operating space" where a vegetative community can grow and adapt to site limiting factors that constrain a trees ability to capture and process solar energy to grow biomass, i.e., its productive capacity. Identifying this space begins to identify the bounds and historic range of Total Net Primary Productivity (tNpp) measures for a site, but by itself does not reflect the potential of an ecosystem to grow on a site or how the same ecosystem adapts to disturbances and climate change. It does not allow us to manage or to inform ecosystem managers whether intervention can increase the tNpp or growth at any location under a climate change scenario. In addition, a sites productive capacity needs to be bracketed by its site-specific reference maximum productivity to calculate its "ecosystem fit" to assess a forests vulnerability to climate change. The ecosystem fit is calculated as the measured (tNpp) to the Theoretical Maximum Net Primary Productivity (TNpp) where the TNpp represents the site's capacity for sustained capture and processing of solar energy with no site scale limits to growth or solar capture by trees. The TNpp becomes an internal reference calculated for each site to identify the upper bounds of growth where no internal site factors influence growth. The tNpp is the actual NPP directly measured at each site, and it reflects a realistic lower total NPP achieved due to the site edaphic, climatic, and ecological constraints reducing plant processing solar energy into biomass. Once the specific combination of site factors is identified, the sites safe operating space can be assessed by determining the range of tNpp found in other sites with similar vegetative types, similar soil order and soil textures, as well as climatic conditions. To understand the role of forests in climate change, there is a need to identify the attributes of a local site that makes an ecosystem vulnerable to a loss of productive capacity due to land-uses and/or disturbances, as well as what

makes a forest less fit for its site. A global meta-data of 267 published research studies reported tNpp from mature natural forests and 40 studies from forest plantations was used to test the following hypotheses related to the role of forests in mitigating climate change and how vulnerable forest stands are to climate change: [1A] Using a global scale analysis of direct measures of tNpp to explain forest vulnerability to a loss of productive capacity will identify climatic factors as explaining a greater proportion of the variability in tNpp since this scale of analysis is not sensitive to how forests and their associated symbiotic microorganisms adapt to local scale edaphic and climatic conditions; [1B] Grouping and comparing direct measures of tNpp at the climatic level (i.e., boreal, temperate, tropical) by forest leaf phenology (i.e., evergreen, deciduous) will result in different combinations of climatic and edaphic factors explaining forest tNpp levels depending on how strongly productivity in each group is sensitive to site-level edaphic and climatic constraints; [2] Local scale edaphic and/or climatic factors will not explain the total productive capacity of plantation forests and their vulnerability to climate change since nutrients and/or water limitations to growth are managed by fertilization and/or irrigation so site level edaphic and climatic factors do not control tNpp. The Ecosystem Fit or the tNpp/TNpp (Theoretical Maximum Potential Productivity) ratio can be used to assess how well a forest is able to adapt to its site constraints to grow biomass and whether the forest is vulnerable to climate change because it uses a reference maximum productivity level that is external to the site and not influenced by internal site factors. It may identify which forests are better adapted to their growth environment and which forests may need management intervention.

Key words: ecosystem fit, maximum potential productivity, real NPPt, boreal forests, temperate forests, tropical forests, plantations, soil order, soil texture, climatic factors

1. Introduction

There is little question in the scientific community on the importance of using Total Net Primary Productivity (tNpp) to assess the resilience and vulnerability of terrestrial ecosystems to disturbances. Net Primary Productivity (Npp) measures the efficiency at which energy passes through the primary producers (plants) and maintains the functions of the ecosystem (Vogt et al., 1997), as this energy flows through microbes, fungi, protists, and animals. Two common techniques to estimate or measure tNpp are used: 1) Satellites and models use aboveground parameters, i.e., Absorbed Photosynthetically Active Radiation (APAR) as an integrated estimate of tNpp that combines meteorological constraints and the photosynthetic apparatus, i.e., leaf area, which fixes the carbon (Running & Zhao, 2015) and therefore are sensitive to variables that impact photosynthesis, e.g., temperature and moisture, and for vegetative types with no pre-existing site specific data and thus does not incorporate the mechanistic relationships that explain how trees adapt to their growth environment, and over larger geographic scales, and 2) Field-based collection of climatic, edaphic, and biomass at the stand level or smaller scale.

1.1. Direct and Indirect Estimates of Forest Productive Capacity

Direct measures of tNpp requires data on multiple variables that are typically not collected by research projects using the following equation: Net Primary Production = change in annual plant biomass [leaves, stems, coarse roots, fine roots] + losses of that year's biomass during the year [detrital inputs (litterfall) + root turnover and decomposition, mycorrhizal growth, consumption by animals + other losses (organic acids, leaf exudates, root exudates, root turnover, mycorrhizas) (Grier et al., 1981; Vogt et al., 1997). Most studies do not measure all the components of the tNpp

since methods may not exist that can be utilized in the field and measuring each component of the equation is costly and time consuming.

Global carbon budgets need accurate NPP models (Gower et al., 1999) including belowground NPP estimates. The scientific community continue to disagree on how to measure tNpp and all its pools and fluxes, especially the belowground Npp (bNpp) (e.g., Grier et al., 1981; Gower et al., 1999; Clark et al., 2001; Geider et al., 2001; Battipaglia et al., 2020). These disagreements focus on the tools and methods to measure variables that vary spatially and temporarily. Despite researchers being able to see aboveground parts of trees, there are high errors in the quantification of aboveground biomass depending on the methods used to measure it and how the data are scaled from site level studies to remotely sensed data (Temesgen et al., 2015; Gonçalves et al., 2017; Xu et al., 2018). Before the late 1970s, NPP was measured using above- and below-ground biomass since the perception was that the above- and belowground parts of a plant responded to disturbances or manipulations in a similar manner as well as biomass studies showed that less than 30% of the total biomass of a plant was belowground. However, in the 1980s, many ecosystem-based studies began to be published showing that even though fine roots contributed less than 2% of the total plant biomass that up to 40% of total plant NPP may occur in this root and mycorrhizal pool (Vogt et al., 1997). Researchers acknowledge there are many sampling errors for all estimates of changes in above- and belowground productivity (Clark et al., 2001) and direct and indirect estimates of the aboveground (aNpp) and bNpp (Gower et al., 1999), especially when the belowground is an invisible system so changes in structures and functions are not readily observed.

Whether bNpp is directly measured or estimated using models, the fine root NPP estimates produced using both methods vary significantly. This stimulated research to identify the upper bounds of how much carbon is potentially available to grow roots and mycorrhizas using indirect

methods to calculate annual gross carbon budgets for forest stands due to insufficient data availability from representative global forest sites. The indirect method Ryan (1991) developed was not designed to replace direct measures of GPP but to validate direct measures of carbon fluxes in forests and identify the upper threshold of total potential C fluxes, especially in sites with no data. Ryan (1991) compared direct measures of total (above- and belowground biomass) annual carbon flux for a 23- and 180-year-old Pacific silver fir (Grier et al., 1981) and compared the field data with the results generated using the Forest-BGC model. The Forest-BGC model estimated bNpp using the empirical relationships developed between soil respiration and litterfall by Raich and Nadelhoffer (1989) – called the Soil Carbon Budget model - using data from 30 forest sites. When Ryan (1991) compared the direct measures of NPP and C flux using the Forest-BGC model, the direct bNpp estimates were twice as high as the model estimates. The Soil Carbon Budget model estimated bNpp to be 254 g C m^{-2} yr^{-1} and the Grier et al. (1981) study directly measured it to be 600 g C m^{-2} yr^{-1}. The direct measures of bNpp and mycorrhizas by Grier et al. (1981) and Vogt et al. (1982) reported that mycorrhizas account for 15% of the tNpp and had a significant role in the nutrient cycles in these subalpine forests. The model analysis by Ryan (1991) produced an upper threshold of gross carbon fluxes that suggested direct measures of bNpp overestimate GPP by ~22% in the 180-year-old Pacific silver fir stand. This suggested that direct measures of bNpp were problematic, and a model would provide a more realistic lower upper limit to fine root NPP as well as GPP measures.

The Soil Carbon Budget model developed by Raich and Nadelhoffer (1989) estimates bNpp as the difference between soil respiration and litterfall – two variables that do not consistently drive changes in bNpp (Vogt et al., 1996) (1997?). The same variables that impact soil respiration and litterfall also influence bNpp but are not the primary factors affecting bNpp. For example, soil

respiration is highly sensitive to temperature (Sprugel et al., 1994), aboveground litterfall varies significantly with precipitation and climatic factors (Vogt et al., 1986; Tonin et al., 2017), and fine root NPP is controlled by various factors, e.g., soil chemistry/physics/biology, climatic, genetics, plant age (Vogt et al., 1995; Yuan & Chen, 2010). Vogt et al. (1986) did not find a relationship between annual litterfall and root production but showed that 77 to 88% of the variance in root NPP was explained by the nitrogen content of the litterfall. Do et al. (2015) did produce a significant regression between aboveground litterfall and fine root production in an old-growth evergreen broad-leaved forest in southwestern Japan but the mechanistic relationship was not provided. This suggested that there is not a constant ratio between litterfall and soil respiration that can be used to estimate bNpp. Since litterfall data are more readily available from global forests, it is frequently used to estimate bNpp when root data are not available. Despite Ryan (1991) saying his method should not replace direct measures of bNpp, this indirect method has been readily adopted by researchers to calculate GPP and as a tool to exclude direct measures of bNpp when gross carbon budgets exceeded the upper bound of the estimated GPP or when was not available for a site (e.g., Clark et al., 2001; see references cited in Litton et al., 2007).

It is challenging to obtain realistic values for GPP and plant respiration (Sprugel et al., 1994) which is an important part of GPP estimates. It varies significantly between sites. In the studies analyzed by Litton et al. (2007), they showed from 42-71% of GPP was partitioned to respiration but this variation to respiration did not vary with resource availability. Their study also reported that only 20% of the partitioning to the belowground was based on variance in GPP even though 51 of the 63 experiments they analyzed used the carbon balance technique of Raich and Nadelhoffer (1989) to calculate the total belowground carbon flux. Litton et al. (2007) also suggested their data produced an independent method to estimate GPP since aboveground NPP of foliage (aNppfoliage)

explained 71% of the variance in GPP. Zhang et al. (2018) demonstrated well how the scaling of photosynthesis in subtropical forests varies by successional stage and species trait identities and foliar nutrients limitations of P or N. The use of GPP to produce the bounds of total productivity is limited by it being influenced by site level growth constraints and the lack of a consistent relationship with how much is partitioned into respiration. There is a need to identify another approach to estimate the upper bound of potential productivity at a given site that is not impacted by specific site scale variables developed at one site and therefore do not represent or transfer to other forest stands with different constraints to a forest's productive capacity. Also, GPP is not correlated to the nutrients acquisition by fine roots, mycorrhizas and/or plant genetic factors.

1.2. Maximum potential productivity as the upper bound of site-scale productivity

To understand the impact of climate change on local unmanaged forest ecosystems requires us to be able to identify: 1) the upper and lower thresholds of productivity in a "safe operating space" (SOS) (e.g., Scheffer et al., 2015) and what factor(s) produce(s) these productivity thresholds in the confines of the SOS that determines whether a vegetative community is able to grow and adapt to a diversity of factors and disturbances that limit its productive capacity (Table 4-1); 2) the interactions between factors at each local site that makes it vulnerable to a loss of productive capacity (Vogt et al., 2016) due to land-uses and disturbances; and 3) a reference upper-level threshold of maximum potential productivity for each site that is specific to the site and calculated using variables external to the site so it is not influenced by variable site specific factors (Gordon et al., 1992).

The reference theoretical maximum net primary productivity or potential Npp (TNpp) is derived using external site factors - where tree growth is not limited by any site factors to calculate its "Ecosystem Fit," (tNpp/TNpp) of a forest stand (Gordon et al., 1992). The reference TNpp

expresses how well a forest is adapted to its site, i.e., its fit, and does it have capacity to grow more biomass beyond its current growth rate as suggested by field measures of tNpp. This is where the Ecosystem Fit (Gordon et al., 1992), or reference conditions is important because it compares the real tNpp to a TNpp, or a reference tNpp for that site without the site level constraints that limit growth. Yuan and Chen (2010) summarized well the diversity of soil conditions coupled with climate, stand age, successional stage all influenced fine root dynamics in boreal forests. All the factors they mentioned will decrease the potential growth rate reached at a site. To estimate the TNpp for a site, internal ecosystem factors cannot be used since there are varying combinations of factors that control root dynamics.

Using meta-data of TNpp for global forests to develop a reference tNpp is not realistic since site-level data do not represent the potential of a forest to grow biomass under its specific site limiting growth constraints. Current tNpp does not allow an assessment of how vulnerable a forest is to climate change since a tNpp estimate doesn't rank the forest as being in a low, medium or high tNpp category or how well adapted it is to its site (Vogt et al., 2016).

The continuing challenge to identifying a SOS is the lack of a reference condition that can be used to determine where a forest is functioning within its site-specific constraints to growth. Each site's tNpp measurements does not produce an index of how well trees are or are not growing because of site limiting factors. To compare sites will require the development of an external productivity measure that defines the maximum potential productivity for that site. For forest stand level assessments, an internal reference gross carbon flux would vary in an unpredictable manner and would reflect the variable influence of site-specific factors on productive capacity. This is where calculating the "Ecosystem Fit" (eFit) of an organism to its current environment can be utilized to

calculate a theoretical maximum net primary productivity as a reference condition to assess the potential of any site to grow biomass (Gordon et al., 1992, 1993).

To estimate TNpp we utilized a simple light use efficiency model (Delucia et al., 2014) from eco-physiological principles without water or edaphic limitation. Our model provided a theoretical maximum value for TNpp in natural and managed ecosystems where TNpp was calculated as the product of total incident solar radiation during the active growth period of the year, photosynthetic conversion efficiency at which C3 plants convert absorb solar energy into tissue, interception efficiency at which tree canopies absorb solar radiation, and the inverse of the specific energy of plant biomass. To account for photosynthetic efficiency being strongly temperature dependent, TNpp was calculated from site level measurements of remotely sensed solar radiation and temperature data extracted at each sites' geographic coordinates.

This theoretical maximum net primary productivity potential (TNpp) is not equivalent to GPP estimates which includes plant and soil respiration data when producing a carbon budget and limits the maximum calculated GPP. Therefore, it is not comparable to a maximum theoretical potential NPP. It does not function as a reference to determine how adapted a forest is to its site-level constraints and what factors will cause reductions in total productivity. Gross primary productivity changes in response to environmental and edaphic conditions. The importance of climatic factors in controlling site-scale GPP was shown by Fisher et al. (2007) when they experimentally induced drought stress in an Amazonian rain forest using a throughfall exclusion experiment. This experiment showed that 2 years of continuous rainfall exclusion (50% reduction in throughfall reaching the soil) resulted in a modeled prediction of an average decrease of 13-14% in GPP and 40-45% decrease in GPP during the dry period of the year. Despite this reduced growth, trees appeared able to adapt to large natural disturbances if the temporal and spatial scales are

adequate for them to recover. Gross primary productivity varies with site-scale changes but does not provide a potential maximum productivity for the site.

1.3. Assessing the site-scale constraints to productive capacity

A diverse combination of site scale edaphic, mycorrhizal and climatic factors limits the total productive capacity of a forest under non-disturbance conditions and how well trees are able to adapt and maintain their tNpp with disturbances such as atmospheric nitrogen deposition (Lilleskov et al., 2008) and/or climate change. Forest ecosystem managers need to know these relationships to better select sites to sequester carbon in forest biomass, but this information is frequently not available for most sites. Without this information, it will be challenging to assess how vulnerable a forest is to climate change, what is the potential of the site to sequester carbon, how has pollution impacted the nutrient availability at a site, or what are the lower and upper thresholds of productivity for a given site. Evaluating whether a forest ecosystem is within its acceptable SOS (Scheffer et al., 2015), or to increase the sites productive capacity, will require information on what factors limit tree growth.

Since plants adapt to a diversity of site scale soil physical and chemical characteristics by shifting carbon allocation patterns between the above- and the belowground tree components to mycorrhizal associations, using gross carbon budgets to determine the upper bounds of bNpp levels for a site does not capture the multiple adaptive processes that explain these allocation shifts or which combination of site-level factors are early warning indicators of changes in tNpp. Many of the responses to environmental change occur belowground and are early warning and sensitive indicators to system level changes that are not visible or detected aboveground (Vogt et al., 1993).

The vulnerability of forests to a loss of productive capacity in response to climate change needs to measure the part of the ecosystem maintaining a site's productive capacity under a diversity of edaphic and climatic factors. The vulnerability of trees to a loss of productive capacity is most limited by soil nutrients and a soil's ability to store water. In the Vogt et al. (2016) study, tropical forests growing on sandy loam and clay soil textures reached tNpp levels in the Low and Medium tNpp grouping whereas the forests reaching Medium and High tNpp levels were found growing in sand-textured soils. To photosynthesize, leaves of trees need nutrients mostly derived from soils to produce the enzymes to convert CO2 to complex carbon compounds. This suggested that the current structure of a global carbon model is good at estimating how much carbon or biomass can potentially accumulate in a region or globally but are not sensitive to assess how forests adapt to their site limiting constraints to growth. These models do not include edaphic factors controlling carbon allocation to roots and mycorrhizas since direct measurements of these variables and relationships data are not available to parameterize and validate the models. Direct measures of tNpp also need to be used to calculate the lower and upper threshold of tNpp using the physical and chemical characteristics of soils.

1.4. Ecosystem fit can inform forest resilience in the face of climate instability

An understanding of how forests adapt to changing environments is fundamental for assessing their ecological dynamics and predicting their resilience to climate change. This research study used eFit and the SOS of each climatic region to gauge the vulnerability of forest ecosystems in each climate. Ecosystem Fit reflects the potential of a site to grow biomass and allows a determination of how far the measured tNpp is from its potential maximum. This shifts prediction of forest carbon budgets at regional or global scales to factors in a forests' eco-physiological and evolutionary functions (Gordon et al., 1992). The eFit of the current ecosystem to the site was

shown by Gordon et al. (1993) to be much lower with an ecosystem reaching 25.6% or less of what a calculation based on factors external to the site. Most plants do not reach their genetic potential for productivity because of the environmental stresses they experience (Geiger & Servaites, 1991). Even crops growing under high-intensive management regimes only reach 30% of the yields that the genetics of the plants would suggest that a plant should reach (Boyer, 1982). This suggests that factors internal to the site (e.g., tree species and their genetics, development stage of the soils, which symbionts are found on the roots, pollutants, management practices or land uses) reduce the capacity of photosynthesizers to fix higher amounts of carbon. This is akin to the trade-offs realized between natural selection and sexual selection where extreme phenotypes are tamped down by the increased risk of predation or lack of foraging efficiency (Rieseberg et al., 2002).

Ecosystem fit would have better predicted the differential recovery rate of forests following a mega-drought that occurred in the Amazon in 2005 (NASA, 2013). NASA detected half of the forests impacted by the 2005 drought had not recovered by 2010. Potts (2003) reported how droughts in northwest Borneo resulted in higher rates of tree mortality when growing on soils with sandy loam texture compared to clay soils.

Once the reference condition is determined, it will be important to identify which combination of variables drive how forests adapt to their environment within the SOS. Since most field-based studies are conducted during non-disturbance periods, carbon allocation shifts can be used to isolate the combinations of climatic and soil factors that will determine what the lower and upper threshold of productivity are for any location during the intervals between major disturbance events.

We used a global scale analysis of direct measures of tNpp and a calculated eFit to explain forest productivity and vulnerability to a loss of productive capacity with a non-linear multivariate

approach at two spatio-ecophysiological scales; global phenological traits and regional climatic zones. The aim of this study was to determine the structure of natural forest and to test how the groupings were related to site-level environmental variables and site-independent growth parameters to assess the adaptive productive capacity of forests to grow biomass. To achieve this, we compared direct measures of environmental conditions at 267 natural forest sites to their modeled site-independent safe operating space to identify thresholds of adaptive capacity to grow and the limits currently experienced.

The following hypotheses were tested:

[1] Global-scale leaf phenological traits are independent of productivity and eFit and are therefore not determinative of primary productivity nor sensitive to local environmental constraints.

[2] Climatic conditions are modulated by edaphic factors and will explain tNpp and eFit depending on how sensitive the response in each group is to region-level environmental constraints.

[3] The "safe operating space" at each site, the upper and lower bounds of environmental conditions, will identify which forests are better adapted to their current growth environment and may identify which forests are more vulnerable to climate change.

Comparison of data from individual sites were analyzed using multivariate statistical methods. Techniques were applied that accounted for unbalanced data and non-parametric variables. Binary division of sample populations was used to create regression tree models, to determine the correlations and thresholds of productivity. Objectives of this study were to: (i) identify the upper and lower productivity thresholds and Safe Operating Space for forests grouped by climatic zones, and (ii) use field collected tNpp data and site factors to show the diverse combinations of variables that predict tNpp at the stand level in boreal, temperate, and tropical forests.

2. Methods

2.1. Database creation and description

These analyses were based on a data set of field measurements of tNpp, with climatic variables of annual-based air temperatures and precipitation, elevation, and edaphic conditions including soil type (i.e., taxonomic soil orders) and soil texture (USDA soil taxonomy system) gathered from 307 published plot-level sites (Table 4-1).

The dataset consisted of 267 natural forest sites and 40 plantations. We characterized the natural forest sites at two spatio-ecological scales. One was at the large-scale using parameters of leaf phenology consisting of evergreen forests (n = 191) and deciduous forest (n = 76), and (n = 76), and the second was at the meso-scale with parameters of climatic regions consisting of boreal (n = 70 with 87% evergreen), temperate ((n = 103 with 64% evergreen), and tropical (n = 94 with 68% evergreen). We further evaluated tropical forests by region and management: Region 1 = Americas with 67 natural forest sites with (85% evergreen) and 5 forest plantation sites (100% evergreen); and Region 2 = Asia with 23 natural forest sites (22% evergreen) and 28 forest plantation sites (57% evergreen). The sites were globally distributed between the 39th parallel south to the 67th parallel north (Fig. 4-1).

Table 4-1: Variables used in our database.

Variable	Units
Global regions	1) America; 2) Asia; 3) Africa; 4) Europe; 5) New Zealand-Australia
Air temperature (˚C)	Mean, minimum, maximum annual temperature, grow temperature (mean temperature during grow season)
Precipitation (mm/yr)	Millimeters
Elevation (m)	Meters above sea level
Soil texture; standard class matrices based on proportion of sand, silt, clay (USDA Soil Taxonomy, 1999)	1 = sand; 2 = loamy sand; 3 = sandy loam; 4 = fine sandy loam; 5 = very fine sandy loam; 6 = loam; 7 = silt loam; 8 = silt; 9 = sandy clay loam; 10 = silty clay loam; 11 = clay loam; 12 = sandy clay; 13 = silty clay; 14 = clay; 15 = peat (*not texture*)
Soil orders in the US Soil Taxonomy system (USDA Soil Taxonomy, 1999)	1 = Mollisols (*none present*); 2 = Alfisols; 3 = Andisols; 4 = Histosols; 5 = Inceptisols; 6 = Ultisols; 7 = Entisols; 8 = Spodosols; 9 = Oxisols; 10 = Aridisols; 11 = Vertisols; 12 = Gelisols
Allocation (%)	Belowground bNpp / Total NPP (tNpp) * 100
Total net primary productivity ($Mg\ ha^{-1}\ yr^{-1}$)	Aboveground aNpp + Belowground bNpp = Total NPP (tNpp)
Theoretical maximum net primary productivity (TNpp) ($Mg\ ha^{-1}\ yr^{-1}$)	TNpp = Solar radiation * photosynthetic efficiency * interception efficiency * inverse of biomass * absorption efficiency
Ecosystem fit ratio	tNpp / TNPP

* https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_053588

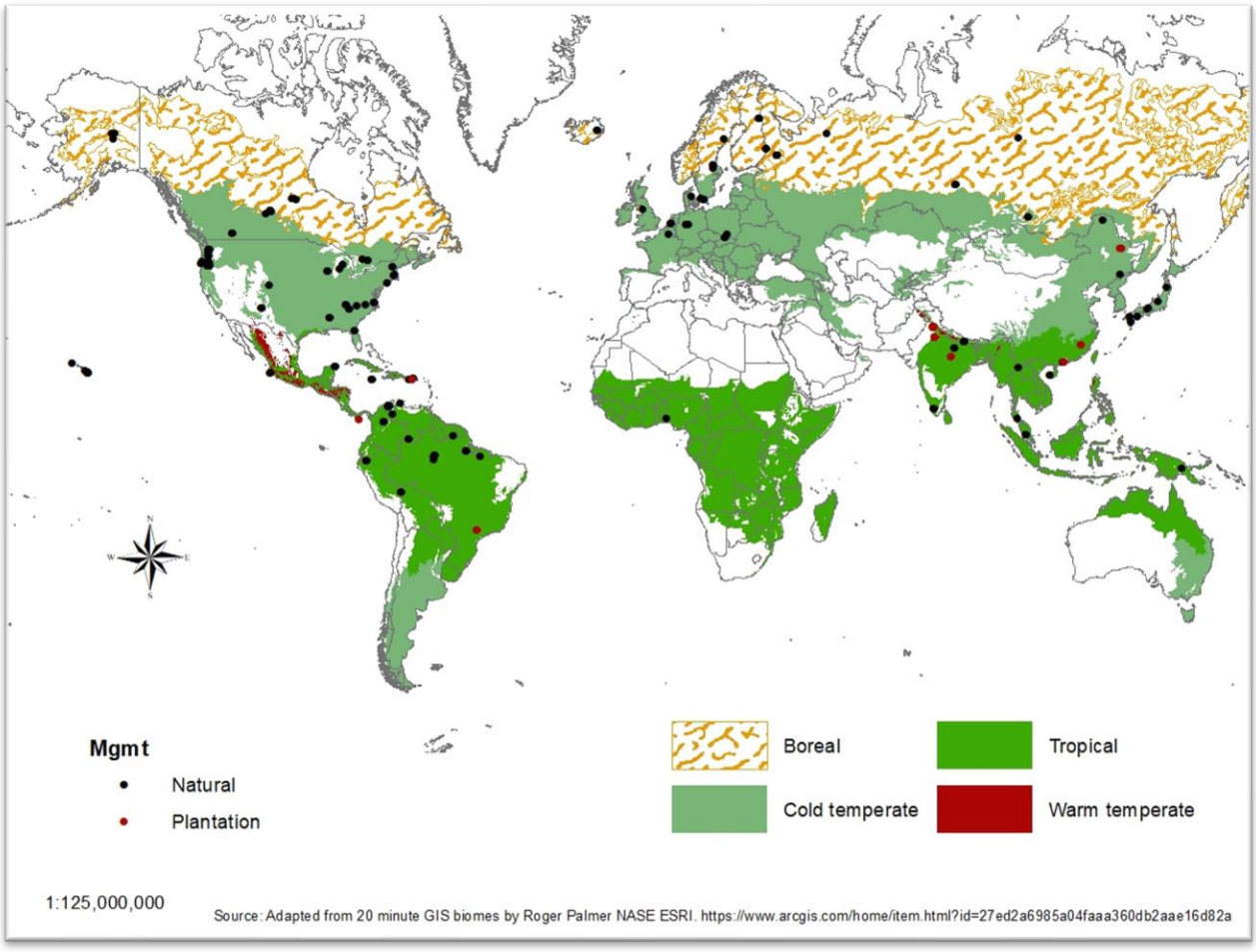


Figure 4-1. Geographic distribution of the forest sites where total net primary productivity was measured with climatic classifications.

Temperate, tropical, and boreal forests have distinct seasonal climate shifts, but in this study, we considered the annual mean, minimum and maximum air temperature, and total annual precipitation as continuous variables to tease out important environmental thresholds of productivity. Generally, tropical forests were located between the equator and the 29th parallel, temperate forests were located between the 30th and 50th parallel, and boreal forests above the 60th parallel. High spatial resolution average monthly temperature data and average monthly solar radiation data (Fick & Hijmans, 2017) were downloaded

[https://www.worldclim.org/data/bioclim.html] and extracted at the geographic coordinates of each forest site and used to derive theoretical metrics of maximum productivity, photosynthetic efficiency and ecosystem fit.

2.2. Calculation of theoretical maximum net primary productivity (TNpp) and Ecosystem Fit (eFit)

To calculate eFit we first modeled the theoretical maximum net primary productivity (TNpp) using a basic light-use efficiency model for the grow season. The model was calculated as the product of mean solar radiation (Fick & Hijmans, 2017), interception efficiency (E_i), the efficiency which forest canopies absorb solar radiation, and the conversion efficiency (E_c), or the rate at which solar radiation that is absorbed by C3 plants is converted into biomass (*sensu* Delucia et al., 2014). Mathematical equations and model parameter specifications were from (Bernacchi et al., 2003).

The calculation of E_c used the average annual atmospheric CO_2 concentration the year of data collection (1981-2011) as measured by the Mauna Loa Observatory (https://www.sealevel.info/co2.html). Model assumptions were that photorespiration was a constant fraction of photosynthesis, photosynthetically active radiation (PAR) was 45% of total solar radiation, and forest canopies absorbed 90% of incoming PAR during active growth. In addition, we assumed no nutrient or water limitations to growth. The growing season was calculated as total month-days when mean monthly temperature surpassed zero (Running et al., 2015).

Photosynthesis is the framework of the ecophysiology of carbon assimilation in forest ecosystems and is an indicator for quantifying the functional limitations imposed by the environment (Gordon et al., 1992). Trees convert solar energy into biomass within their genetic and environmental constraints, so if all the structural and functional attributes of trees are operating at

their optimal levels, and the environmental conditions are within the normal range of operation, then productivity will be maximum for that ecosystem under the conditions present (Gordon et al., 1983). Based on these constructs, eFit is described as a ratio of measured tNpp to a predicted TNpp. Statistical functions were performed in R version 4.0.3 (R Core Team, 2020) and all analyses were subjected to the appropriate tests of normality and behavior of the model residuals.

2.3. Comparing the response with coefficient of the range

The coefficient of the range (CV_r) is the ratio of the difference of the response to the sum of the maximum and minimum values of the response, i.e., a relative measure of dispersion, aka the range coefficient of dispersion. A CV_r was calculated for tNpp and eFit to evaluate the amount of variation among each response in each climate (higher values = greater variation, lower values = less variation). This metric is an index of variability among forests which cannot be compared directly. For example, the range of tNpp in boreal forests was 16.8 Mg ha^{-1} yr^{-1} and in the tropics was 41.9 Mg ha^{-1} yr^{-1}. It seemed that there is greater dispersion in the tropics, but this is not true. The range in the tropics was for more observations (n = 94) than the range in the boreal (n = 70), therefore they cannot be compared directly as their base was not the same. When we converted these two values into CVr we see that the tropics (CV_r = 0.84) had a lower value than the boreal (CV_r = 0.86). Thus, there is less dispersion or variation in the tropics. The opposite was true for eFit indicating that boreal forests (CV_r = 0.83) have less variability than tropical forests (CV_r = 0.87).

2.4. Reduction of factor level complexity

To reduce the complexity of our multi-factor level data, one-hot encoding (dummy variables) was used for our multi-level soil factors. We then applied a three-step procedure for variable selection based on the R packages "`randomForest`" (RF) (Liaw & Wiene, 2002) and

"randomForestSRC" (Ishwaran & Kogalur, 2007; 2021). The complexity of the dataset was further reduced with the versatile package "VSURF" (Genuer, 2019) to: 1) eliminate the irrelevant variables from the dataset; 2) identify the variables related directly to each response for interpretation purposes; and 3) finalize the selection by eliminating redundancy among the variables selected by the second step, for prediction purposes (model simplification). The variables identified were then compared by this process to the variables selected by a RF model built on all variables. The most effective sets of variables were selected based on the lowest RMSE (root mean square error) value and MAE (mean absolute error).

2.5. Gaining insight into our data with clustering

To understand the underlying structure of the data from the selected variables we excluded all phenological and climatic classifiers as they were highly correlated with group membership, and dissimilarities (distances between all pairs of data points) were calculated for the explanatory variables. We applied a new RF method for unsupervised clustering with the function "sidClustering." The first step was "sidification" of the features or staggering the features to have mutually exclusive ranges (Mantero & Ishwaran, 2020), and then forming all pairwise interactions. A multivariate RF model able to handle both continuous and categorical variables was used to predict the SID main features. This method is adept at finding clusters arising from categorical and continuous variables and retains all the important advantages of RF. The processed data were aggregated with the PAM (partitioning around medoids) method which iterates over and over until the medoids don't change their positions. The medoid of a cluster is a member of the cluster which is representative of the median of all the attributes under consideration. The optimal number of clusters were selected by evaluating the average silhouette width and the variance explained by the first two principal components. Silhouette width measures the similarity of each

point to its cluster and compares that to the similarity of the point with the closest neighboring cluster. This metric ranges between -1 to 1, where a higher value implies better similarity of the points to their clusters. The clusters were visualized with nonlinear dimensional reduction with package "`Rtsne`" (Krijthe, 2015) utilizing the t-distributed stochastic neighbor embedding technique (t-SNE) (Maaten & Hinton, 2008).

2.6. Analysis of site parameters with the random forest classification

The results of the cluster analysis were compared to a RF classification model for congruence. Classification predictions with RF are very accurate due an ensemble method that fits many classifications of trees (`ntree`) resampled by bootstrapping based on a random selection of predictors (`mtry`) resulting in the average of multiple classification trees. The trees were aggregated back together, and the model polled the data to assign them to a category (node). The Gini criterion is commonly used to measure node impurity for categorical response variables, or the decrease in node impurity (0 = a pure node). The index can be obtained by: Gini index = $\sum_{j=1}^{r} P_j^2$,

where P_j is a relative frequency of class j in a node.

Variable importance identifies the most influential predictors. At each tree split the variable that contributed to the importance of the impurity measure is stored and as the reduction of the impurity measure accumulates a measure of relative importance of the variables is assigned. Another measure of importance is the decrease in prediction accuracy over all trees were predictor values from the "out-of-bag" (OOB) samples are permuted at every tree.

2.7. Analysis of tNpp and ecosystem fit to site parameters with the random forest regression model

The importance of environmental, climatic and soil characteristics was analyzed with a random forest (RF) regression tree model (Breiman, 2001). Random Forests fits decision trees according to hierarchical levels. First it combined large sets of decision trees where each tree was based on random bootstrap samples that partitioned the data into successively more homogeneous subsets by selecting the most discriminative predictors to improve the regression analysis. The partitioning was repeated until the nodes were homogeneous enough to be terminal. Thus, the prediction was made on the average of many trees rather than a single tree. There was no "pruning" so trees are deep and the randomization in the growing process accounted for any autocorrelation between variables. This means that ecologically meaningful groups were identified when a simple threshold is met. Random forest is a preferred method as it is not affected by violations of the typical model assumptions of linearity and normality with little/no multicollinearity, no auto-correlation, and homoscedastic variance. This method has several desirable characteristics: (i) the ability to handle multiple types of data; (ii) the ability to assess interactions and nonlinearity; (iii) the ability to calculate variable importance, and iv) the ability to handle unbalanced data sets (Lemon et al., 2003).

Instead of an overly complex model (high variance) or an overly simplistic model (high bias) RF decorrelates trees generated by bootstrap aggregating (bagging) and then reduces the variance by averaging them. Typically, about 70% of the original observations are used for the prediction and the remaining 30% are the OOB observations. As OOB predictions are not used in building the trees this part of the process was essentially cross-validation of the prediction estimates. In contrast

to other statistical methods such as AIC, RF measures the importance of a variable directly using the misclassification rate from OOB observations (Cutler et al., 2007).

Although decorrelation of trees in RF protects against multi-collinearity of predictors, one possible limitation is unreliable determination of variable importance with highly correlated predictors (Strobl et al., 2008). Our temperature variables were moderately correlated but each of these variables were necessary to include in the model as each have significant, and different, influences on plant growth. A cross-validation analysis indicated no increase in error when all temperature variables were included compared to using each variable independently. Despite this finding, the standard RF algorithm was inconsistent in assigning the top variable and thus was unable to overcome variable selection bias. More weight is given to correlated predictors as the permutation test is testing that the variable is independent of the response as well as of all other predictors. As correlated temperature predictors are not independent, we get interchangeable high importance scores for correlated variables (Strobl et al., 2009). Due to this limitation we tested an unbiased random forest algorithm, conditional RF `(cRF)` with "`cforest`" function (Strobl et al., 2009) for conditional inference trees in the R package "`party`" (Hothorn et al., 2006). This algorithm performed a permutation test conditioned on the correlated predictors to guarantee unbiased variable selection and variable importance. This study found no difference in the variable selection pattern, so continued with the RF model.

We sought to understand how the interactions between climatic and edaphic conditions modulated the response over environmental gradients and to identify the threshold of continuous variables and unique soil conditions that produced significant changes in forest ecophysiology. To account for the large number of factor levels of soil order and soil texture we generated dummy variables to avoid model failure. This allowed us to identify breakpoints (locations where two

consecutive segments diverge) of variables and individual soil conditions that produced significant changes in the response at each scale.

2.8. Predicting productivity and ecosystem fit

In general, we utilized our mathematical models for three specific purposes: (i) to identify the structure and patterns of our response and important explanatory variables, (ii) to make inferences; a) without prediction - about the relationship between our response and the climatic, environmental and edaphic variables measured at each site, and b) with prediction - to validate the accuracy of our models and understand how forests may respond to environmental change, and (iii) to derive a working conceptual/narrative application (e.g., "tell a story about tNpp and eFit in relation to climate change").

The statistical analysis was carried out in six steps: (1) data mining and one-hot encoding, (2) model building, comparison, and selection, (3) model hyperparameter tuning, (4) identification of the most important variables, (5) validation of model accuracy by regressing observed values on predictions to compare slope and intercept parameters on the 1:1 line, and (6) quantification of the predictions over the marginal effects of the most important explanatory variables.

2.9. Inference from partial dependence plots

Ecological data are often poorly represented by linear models but when it is appropriate, we evaluate these models by examining their coefficients. Complex machine learning models like RF do not provide straight-forward model summaries making their interpretation more abstract. To better assess the results of RF models, partial dependence plots (PDPs) were proposed (Friedman, 2001). Starting with a picture is a simple and powerful interpretive tool as plots provide an intuitive summary of variable characteristics to foster ecological inferences. Partial dependence plots are

based on predictions generated by the RF model and are interpreted by the shape of the dependence line across the range of the predictor variable. In part, the ability of RF models to capture this response behavior is an integral component of their superior prediction value. Intuitively, PDPs can be interpreted as the expected response as a function of the target variable. The slopes correspond to the model parameters and therefore may be viewed as graphical extensions of the coefficients in a linear model. This study used PDPs to understand how forest ecosystems respond to environmental gradients and to identify low-order interactions that are often critical to understanding ecological processes. To avoid overinterpretation we plotted a rug of the data on the axes.

2.10. Random Forest model forest classification predictions

To determine if we could accurately predict forest climatic region, a multi-label classification algorithm was applied. The R package "`caret`" was used to train the model parameters (Kuhn, 2020) and applied a repeated K-fold cross-validation method with ten repetitions (repeats = 10) with the function "`trainControl`" which uses resampling to evaluate the effect of tuning model parameters to optimize model performance across all parameters.

2.11. The "safe operating space" and ecological plasticity

The line behavior of the PDPs observed (i.e., the shape of the prediction line) were categorized into four classes of dependence: (i) hard saturation or no dependence, a straight line (hs), (ii) positive monotonic dependence or an increase of the response (+), (iii) negative monotonic dependence or a decrease in the response (-), and (iv) nonmonotonic dependence or a change in the sign (+/-) of the slope of the response, i.e., unusual curves over the range of explanatory variable (nm). This study evaluated the dependence criteria for each important environmental variable to

identify the SOS where productive dependence / adaptive response was exhibited for that forest ecosystem. This does not mean that productive capacity was limited to the SOS but that there may be more than one strategy for coping with environmental heterogeneity for the environmental condition. For example, if predictions were completely saturated for mean annual temperature, there was no variation of the response across the entire range of mean annual temperature for that group of forests. Therefore, forests showed no dependence on mean annual temperature. Conversely, if forests were strongly dependent on minimum annual temperature, this meant that there were adaptive strategies to cope with a variety of minimum annual temperatures across the climatic region. For PDP interpretive example see Supplemental Information (SI.5). We visualized the RF models as binary trees with the R package "`partykit`" (Torsten Hothorn & Zeileis, 2015).

Physiological traits respond more rapidly to environmental change. We analyzed the plasticity of four physiological responses: (i) tNpp, (ii) E_c, (iii) allocation to below ground tNpp (AbNpp), and (iv) eFit. This summarized the means of important explanatory variables at two threshold levels identified by RF modeling to have strong explanatory power. A threshold of an explanatory variable represents a point along that variables' gradient where the response shows strong directional dependence and a significant change in value, i.e., "an elbow." We interpreted the ecosystem location within the SOS as an indicator of forest resilience.

2.12. Mapping Ecosystem Properties and Effect Traits

Biological traits are attributable to individual organisms so to make inferences about entire ecosystems as functional units we need to scale-up. The problem of scale and higher levels of biological organizational is an important component of community ecology, but the terminology has been ad hoc and confusing among researchers (Field & Ehleringer, 1993; Duarte et al., 1995; Jones & Lawton, 1995). In this study, we adopted the convention proposed by Violle et al. (2007) to

define "ecosystem properties" as any feature or process measured at the ecosystem level such as primary productivity or any other biogeochemical processes. This allowed us to utilize data from populations with unmeasured individual-based traits within ecological communities of unknown demographics.

According to the biomass-ratio hypothesis (Grime, 1998) ecological function will be overwhelming controlled by the traits and functional diversity of the dominant plants of a community, and further this property is relatively insensitive to the richness of subordinate and transient species. Evidence suggests this hypothesis holds for principle biogeochemical processes such as above ground primary productivity (Garnier et al., 2004). Therefore the magnitude of the ecosystem properties measured or derived will be dependent upon the associated effect traits (Lavorel & Garnier, 2002) of the most locally abundant species. Ecosystem properties, therefore, depend on effect traits weighted to the most abundant trees of a forest. Our assumptions in this study were that our response metrics were representative of the dominant tree species with a life history defined by leaf phenology and climatic zone.

There is debate on how to measure phenotypic plasticity and there are many different indexes that have been used, however, most of these indices are context-dependent and do not allow standardization or comparison across different gradients (Sánchez-Gómez et al., 2006; Valladares et al., 2006). A concept that places phenotypic plasticity in the context of a genotype-specific response is called the "norm of reaction" or "reaction norm" (NoR).

A reaction norm represents the suite of non-evolutionary plasticity responses (or no response) commonly observed over the range of an environmental gradient (Pigliucci et al., 2006) (*originally in* Schlichting and Pigliucci 1998). Here we extend generalized NoRs used for populations and microevolutionary inferences, to generalize to macro-eco-physiological inferences at a higher

taxonomic level. We considered general forest characteristics of leaf phenology and compared tNpp, Ec, AbNpp and eFit to the most important environmental variables.

We evaluated the NoRs by plotting the environmental variables across the x-axis and tracking the phenotypic values of the response across the y-axis (tNpp, Ec, AbNpp, and eFit). The two lines represent the soft trait of phenology for each climate zone. The endpoints of each curve is the mean of many measurements. A flat line indicates no effect trait plasticity; otherwise, the value of an effect trait shows environmental dependence.

We can quantify NoRs by plotting the mean of each response at two threshold values and connecting them by a line across the environmental gradient. The response can then be interpreted by the orientation and relationship of the respective lines. The position along the y-axes tells us the effect of phenology and the slope tells us the effect of the environment. If the lines are non-parallel or cross there is an interaction between phenology and the environment. Crossing interactions indicate a range of environmental conditions where deciduous is "bigger" than evergreen, where both intersect and where evergreen is "bigger" than deciduous. Thus, by observing the NoR trajectories we can understand how ecosystem properties depend upon the environment in different climates. This method may serve as an indicator of ecological resilience in the broadest sense.

Norms of reaction are generalizations of the "trait space" of forest ecosystems that permits inference about long-term resilience in a macro-evolutionary context. The topological properties of each set of NoRs provide insight into the nature of the effect trait-environment interactions unique in each climatic zone. For example, forests adapted at high levels of resilience perform within a narrow range of high average annual temperatures (20–28 ˚C) but are intolerant to variability beyond 0-5 ˚C throughout the year (Anjos & De Toledo, 2018).

2.13. Environmental heterogeneity drives adaptation

Tropical forest are more diverse and more ecologically specialized communities of trees where intricate resource/niche partitioning tends to reduce inter-specific competition and low abundance tends to reduce intra-specific competition. The environment is stable, and growth is possible year-round. In contrast, environmental variation has produced a wonderful array of adaptations in more demanding climates and phenotypic plasticity is one of those adaptations. Plasticity may be temporal (seasonality), environmental condition (drought), or ecological (competition, herbivory, or stress from pests). Forests are not limited to phenotypically plastic effect traits - they may use other strategies such as dormancy, plasticity to produce relatively fit (i.e., fitness) phenotypes for alternative conditions, intermediate phenotypes (generalization) and bet-hedging, both of which reduce variance in performance across environments.

Traditionally, evolutionary ecologists define and contrast four strategies for coping with environmental heterogeneity. (1) Specialization: one phenotype is produced that is optimal for a given environment, even though the specialist may find itself sometimes in alternative environments. (2) Generalization: an intermediate, or otherwise general-purpose phenotype is produced, which is at least moderately successful in most environments. (3) Bet-hedging: an organism produces either (i) several phenotypes (e.g., among units in modular organisms or through producing diversified offspring) or (ii) single phenotypes probabilistically. (4) Phenotypic plasticity: environmental factors trigger production of alternative phenotypes. The distinction between these strategies comes down to whether an organism adopts a single phenotype (specialists and generalists) or variable phenotypes (plasticity and bet-hedging). Plastic strategists produce variant phenotypes based on the nature of the environment, whereas bet-hedging strategists produce phenotypic variation within single environments. What is strategic about bet-hedging is that it reduces variance in fitness across

generations, and hence increases geometric mean fitness (Dempster, 1955; Lewontin & Cohen, 1969).

The clearest generality from this type of modeling is that plasticity, in the absence of constraints (i.e., perfect plasticity), is always the superior strategy in variable environments. The fact that plasticity is not ubiquitous (evident for all traits in all species) therefore suggests constraints are ubiquitous in natural systems (reviewed in DeWitt et al., 1998). There are numerous reasons that perfect plasticity is non-existent. First, plasticity may be costly (Van Tienderen, 1991; Via et al., 1995), or constrained by developmental range limits, where plasticity cannot produce phenotypic extremes (due to the need to retain flexibility) (DeWitt, 1998), and finally plasticity is limited by the reliability and accuracy of environmental cues used to guide development (Moran, 1992; Getty, 1996; Tufto, 2000).

The ability to characterize forest effect traits and effect trait plasticity, and their respective dependence on environmental conditions is critical to understanding how forests will respond to the impacts of climate change. Temperature, photoperiod and water availability appear to be the primary drivers of tree dormancy and return to activity in the spring (Delpierre et al., 2016). Over geologic time the annual cycles of growth, reproduction and quiescence have evolved to minimize abiotic stressors (freezing, loss of solar radiation, and low water availability) and to optimize reproductive success the following year. In temperate and boreal forests, the main adaptation is a period of dormancy which shuts down metabolism during the harshest months of the year. We hypothesized that response values would vary over critical environmental gradients, but the response would also differ due to ancient divergent strategies of deciduous or evergreen phenology

Leaf phenological structure is the foundational framework for much of tree natural history. The trait of evergreen and deciduous leaf phenotype is a first order determinant of tree

biogeography and climate is thought to be a primary driver of these distributional patterns (Shivaprakash et al., 2018). Climate change is predicted to alter the selective pressures on tree phenological traits as they struggle to adapt to new conditions. As trees are generally a long-lived species this does not bode well with rapid climate change on the horizon as major adaptive change will need to occur quickly. This could be a problem for organisms with average generation times of decades to centuries (Trouillier et al., 2020). Most leaf phenological traits associated with the annual life cycle of trees are highly responsive to local conditions and highly evolvable (i.e., not tightly coupled to the genome and therefore evolutionary constrained) (Firmat et al., 2017). Leaf phenological behavior is therefore probably not the trait most scrutinized by natural selection nor the most critical aspect for population survival for the immediate future (Firmat et al., 2017).

Given the vastly contrasting selective pressures across the seasons, NoR producing such mismatches would likely be strongly selected against.

3. Results

3.1. Photosynthetic conversion efficiency (Ec) was correlated with high latitude

To identify the large-scale relationship between E_c and latitude we applied Pearson's product-moment correlations to geographic coordinates. At the global scale we identified a significant relationship, $r(305) = 0.78$, $p < 0.001$, with a stronger signal in northern parallels $r(278) = 0.84$, $p < 0.001$. The mean temperature during the grow season was negatively correlated with E_c in northern latitudes $r(246, 30) = -0.99$, $p < 0.001$) and southern latitudes $r(20) = -0.93$, $p < 0.001$. This indicated a compensatory effect, or trade-offs, in high latitudes where environmental forcing favored selection for higher efficiency versus moderate environmental conditions in the tropics (0-

30° latitude) where primary productivity was consistently above the compensation point and E_c was significantly below the base mean (Fig. 4-2).

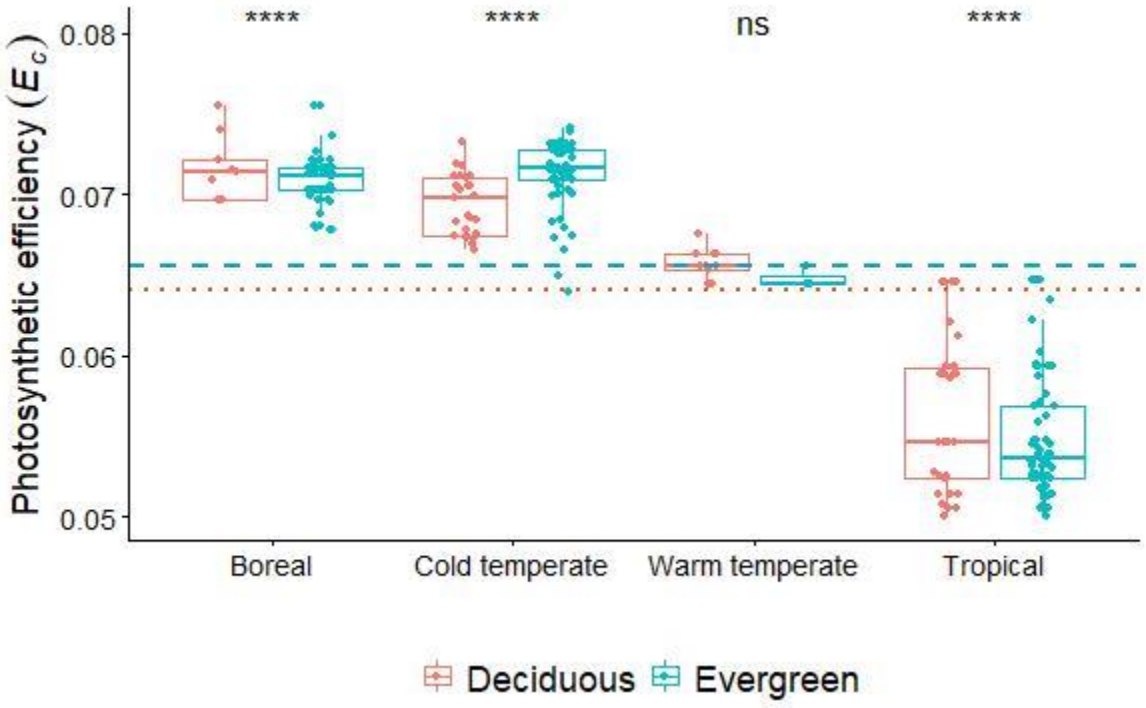


Figure 4-2: Summary of relationships between photosynthetic conversion efficiency (*Ec*), climatic zone and leaf phenology for natural forests. Krustal-Wallis for climate ($H(3)$, 195.8, $p < 0.001$) and phenology ($H(1)$, 8.55, $p = 0.003$) indicates at least one significantly different group. Statistical significance level at top represents multiple pairwise tests against the base mean. Mean represented by a dotted horizontal line for deciduous forests and a dashed horizontal line for evergreen forests. Level of significance; * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$, **** = $p < 0.001$.

There was a fine-scale latitudinal gradient of E_c as we noted differences between cold and warm temperate forests not detected with any other physiological processes measured, bNpp (Fig. 4-3), AbNpp (Fig. 4-4), tNpp (Fig. 4-9) or eFit (Fig. 4-10). For all subsequent analyses warm and cold temperate climate forests were aggregated.

3.2. Descriptive statistics of tNpp and ecosystem fit by climate

The range difference of tNpp in natural forests was 16.8 Mg ha^{-1} yr^{-1} in boreal climates and 39.4 Mg ha^{-1} yr^{-1} in tropical climates with temperate forests intermediate. The largest range difference for eFit was 14.9 % in the temperate climate with the boreal 13 % and the tropics 1.5% (Table 4-2). In this study, tNpp, bNpp, AbNpp, and eFit were analyzed with non-parametric methods to identify the structural dynamics with leaf phenology and environmental conditions.

Table 4-2: Summary statistics for tNpp (Mg ha^{-1} yr^{-1}) and percent ecosystem fit (tNpp/TNPP). Mean ± standard error of the mean (SEM), the range of the trait, and the coefficient of the range of the trait (CV_r) for natural forests by climatic zone.

	tNpp			Ecosystem fit (%)		
Climate	Mean ± SEM	Range	CV_r	Mean ± SEM	Range	CV_r
Boreal (*n* = 70)	07.3 ± 0.48	1.4-18.2	0.86	4.9 ± 0.34	1.3-14.3	0.83
Temperate (*n* = 103)	14.1 ± 0.56	1.3-37.7	0.93	6.4 ± 0.28	0.6-15.5	0.93
Tropical (*n* = 94)	19.3 ± 0.82	4.0-45.9	0.84	7.8 ± 0.39	1.5-21.7	0.87

* Coefficient of the range = (max-min)/(max+min) is a relative measure of dispersion and is based on the difference of the range to the sum range, aka the range coefficient of dispersion. Higher values = greater variation, lower values = more stability.

Interpretation: the range of tNpp in boreal forests is 16.8 Mg ha^{-1} yr^{-1} and in the tropics is 41.9 Mg ha^{-1} yr^{-1}. It seems that there is greater dispersion in the tropics, but this is not true. The range in the tropics is for more observations than the range in the boreal, therefore they cannot be compared directly as their base is not the same. When we convert these two values into coefficients of range (CV_r), we see that the CV_r in the tropics is less than that of the boreal. Thus, there is greater dispersion or variation in the boreal. The opposite is true for ecosystem fit indicating that boreal forests have less variability in ecosystem fit than tropical forests.

3.3. bNpp and below ground allocation differed in extreme climatic zones

We hypothesized that bNpp and AbNpp would be lower in colder climates due to decoupling of primary productivity from the soil. A weaker interdependence would result in lower bNpp and lower AbNpp in the boreal climate which would gradually increase as forests were located closer to the equator. We tested for differences between bNpp by climatic zone and found very strong evidence of a difference between the mean ranks of at least one pair (H (2) = 61.22, p = < 0.001, Kruskal-Wallis). Wilcoxon signed rank pairwise tests carried out for the three pairs of climatic zones, identified strong evidence (p < 0.001, with Bonferroni correction) of lower median bNpp in boreal climates (1.1 Mg ha^{-1} yr^{-1}) and higher median bNpp in tropical forests (6.7 Mg ha^{-1} yr^{-1} (Fig. 4-3).

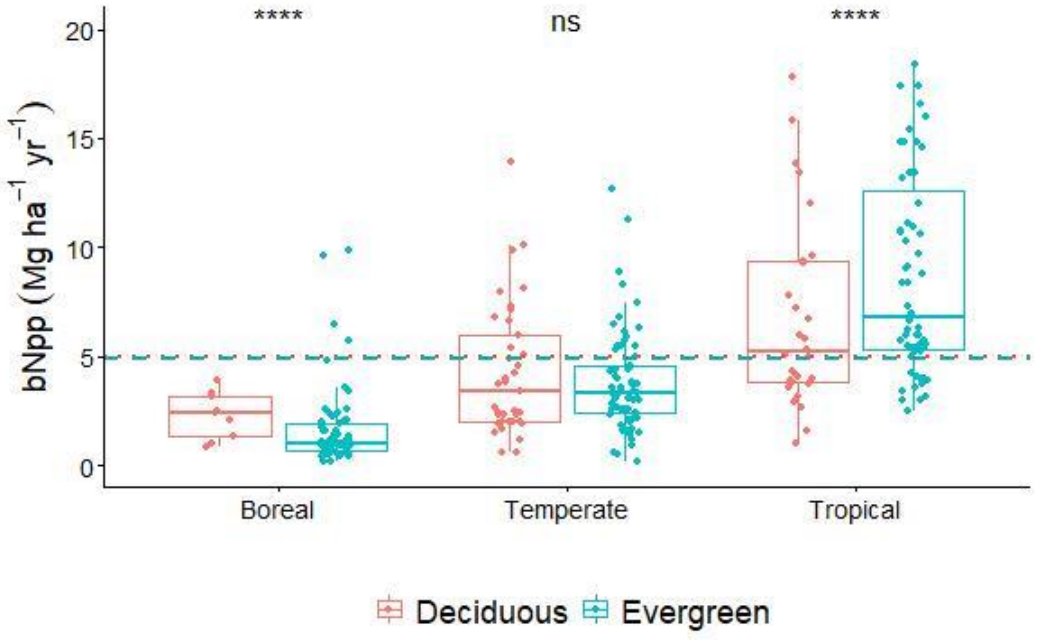


Figure 4-3: Summary of relationships between below ground net primary productivity (bNpp), climatic zone and leaf phenology for natural forests. Krustal-Wallis for climate (H(3), 127.6, p < 0.001) and phenology (H(1), 1.64, p = 0.20) indicated at least one significantly different climatic group. Significance level at top represents multiple pairwise tests against the base mean. Mean represented by a dotted horizontal line for deciduous forests and a dashed horizontal line for evergreen forests. Level of significance; * = p < 0.1, ** = p < 0.05, *** = p < 0.01, **** = p < 0.001.

Comparison of AbNpp identified very strong evidence of a difference between the mean ranks of at least one pair (H (2) = 61.22, $p < 0.001$, Kruskal-Wallis) and Wilcoxon signed rank pairwise tests, carried out for the three pairs of climatic zones, identified strong evidence ($p < 0.001$, with Bonferroni correction) of 16.3% median AbNpp in boreal climates and 43.6% median AbNpp in tropical forests (Fig. 4-4).

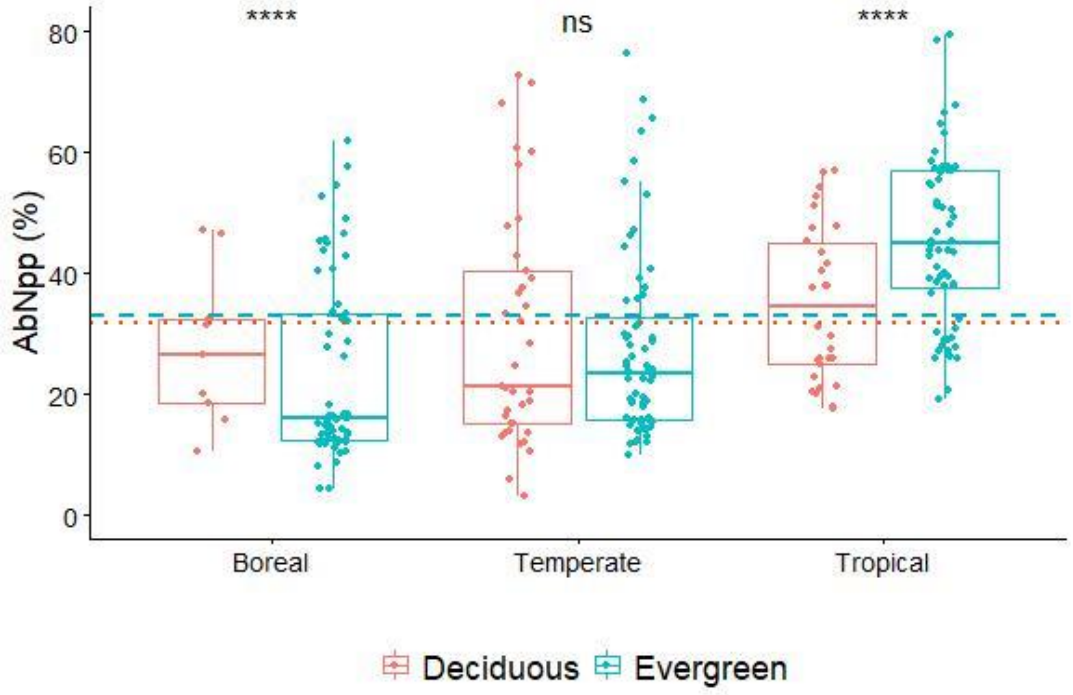


Figure 4-4: Summary of relationships between allocation to bNpp (AbNpp), climatic zone and leaf phenology for natural forests. Krustal-Wallis for climate (H(2), 58.9, p < 0.001) indicated at least one significantly different climatic group and phenology (H(1), 0.005, p = 0.94) was insignificant. Significance level at top represents multiple pairwise tests against the base mean. Mean represented by a dotted horizontal line for deciduous forests and a dashed horizontal line for evergreen forests. Level of significance; * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$, **** = $p < 0.001$.

3.4. Productivity in the tropics differed due to region, management, and soils

To explore our hypothesis that productivity of tropical forests would vary by region (Americas *vs* Asia) and forest management (natural *vs* plantations) multiple comparisons were made. Natural forests showed similar tNpp and eFit across both regions and Asian natural and plantation forests were similar in tNpp and eFit. American plantations were significantly higher (Fig. 4-5).

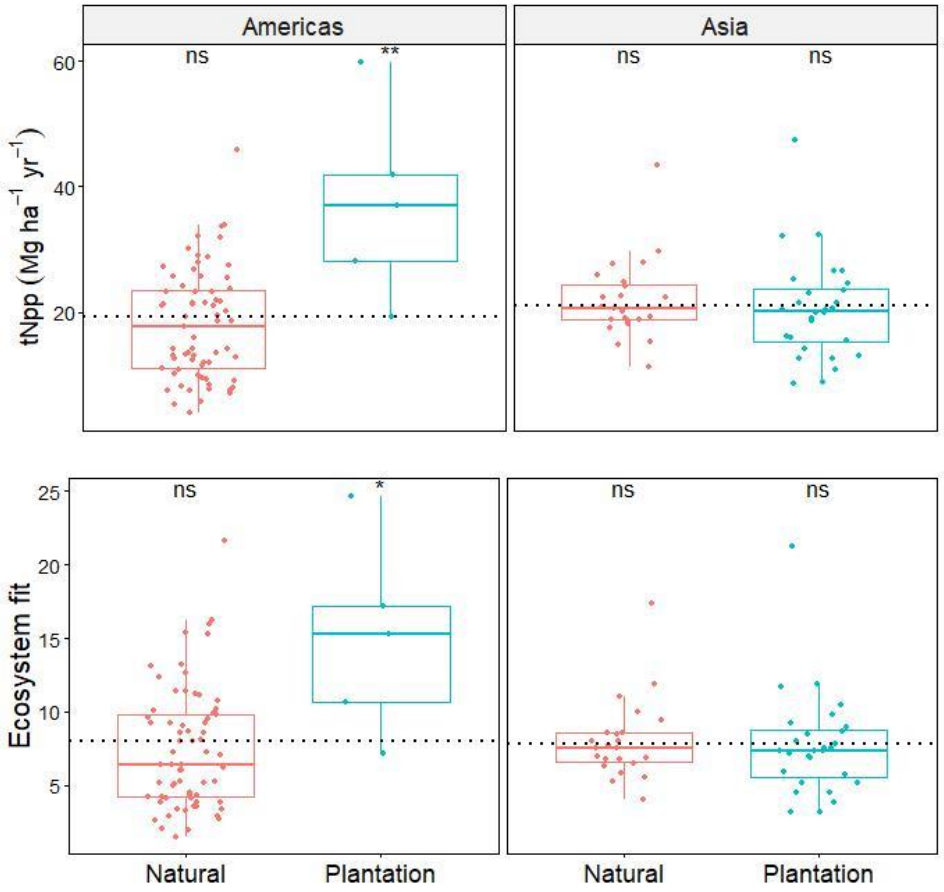


Figure 4-5: Summary results of the comparison of tNpp and ecosystem fit among natural forests and plantations in tropical America and Asia. Wilcoxon rank sum test found moderate differences between the Americas and Asia of tNpp (W = 1549.5, p = 0.07) and no significant difference between regions for ecosystem fit (W = 1752, p = 0.33). Significance level at top represents pairwise tests against the base mean for each region/response. Mean represented by a dotted horizontal line. Level of significance; * = p < 0.1, ** = p < 0.05, *** = p < 0.01, **** = p < 0.001.

A two-way analysis of variance (ANOVA) for unbalanced data sets was conducted on the influence of management and region to tNpp. Management included two levels (natural, i.e., no management vs plantations, i.e., various levels of management) and global region included two levels (Americas *vs* Asia). All effects were statistically significant at $\alpha = 0.05$. The main effect for management yielded an F ratio of $F(1, 119) = 13.89$, $p < 0.001$, indicating a significant difference for natural forests (mean = 18.92, sd = 8.32) and plantations (mean = 22.97, sd = 11.01). The main effect for region yielded an F ratio of $F(1, 119) = 7.06$, $p = 0.008$, Americas (mean = 19.24, sd = 1.039) and Asia (mean = 21.10, sd = 7.39). The interaction effect was significant, $F(1, 119) = 13.09$, $p < 0.001$ (Table 4-3).

Table 4-3: Pairwise marginal means of tNpp. Type III, two-way ANOVA results are given on the log (not the response) scale. P value adjustment: "Tukey" method for comparing a family of 4 estimates. (Natural = natural forests, Plantation = Plantation forests; 1 = forests in Americas, 2 = forests in Asia)

Contrast	Estimate	SE	df	*t* ratio	*p* value
Natural 1 - Plantation 1	-0.789	0.212	119	-3.728	*0.001*
Natural 1 - Natural 2	-0.293	0.110	119	-2.656	*0.044*
Natural 1 - Plantation 2	-0.186	0.103	119	-1.812	0.273
Plantation 1 - Natural 2	0.496	0.225	119	2.201	0.129
Plantation 1 - Plantation 2	0.602	0.222	119	2.719	*0.037*
Natural 2 - Plantation 2	0.107	0.128	119	0.832	0.839

A two-way ANOVA for eFit found all effects statistically significant at $\alpha = 0.05$ except for region. The main effect for management type yielded an F ratio of $F(1, 119) = 10.84$, $p = 0.001$, indicating a significant difference for natural forests (mean = 7.64, sd = 3.82) and plantations (mean = 8.81, sd = 4.79). The interaction effect was significant, $F(1, 119) = 9.71$, $p = 0.002$ (Table 4-4).

Table 4-4: Pairwise marginal means of ecosystem fit. Type III, two-way ANOVA. Results are given on the log (not the response) scale. P value adjustment: "Tukey" method for comparing a family of 4 estimates. (Natural = natural forests, Plantation = Plantation forests; 1 = forests in Americas, 2 = forests in Asia)

Contrast	Estimate	SE	df	*t* ratio	*p* value
Natural 1 - Plantation 1	-0.759	0.231	119	-3.293	*0.007*
Natural 1 - Natural 2	-0.179	0.120	119	-1.484	0.450
Natural 1 - Plantation 2	-0.097	0.112	119	-0.866	0.823
Plantation 1 - Natural 2	0.581	0.246	119	2.367	0.089
Plantation 1 - Plantation 2	0.662	0.242	119	2.743	*0.035*
Natural 2 - Plantation 2	0.082	0.140	119	0.582	0.937

We hypothesized that soil variables would be more important to tropical forests in the Americas due to the predominance of nitrogen-fixing trees in Asia. A RF regression analysis of tNpp indicated precipitation and temperature predominantly explained the variance but elevation and Entisol and Oxisol type soils were indicated as additional explanatory variables (Table 4-5).

Table 4-5: Summary results of RF regression for tNpp in tropical forests.

Model	Variance Explained	RMSE	Std Error	VIMP (w/standardized increase node purity)
Full model	31.5 %	5.14	0.70	MinTemp (1.0), Management (0.58), Precipitation (0.54), Oxisols (0.52), MeanTemp (0.46), MaxTemp (0.40), Elevation (0.33), Entisols (0.28), Region (0.21), GrowTemp (0.21), Texture2 (0.11), Texture3 (0.09)
Americas	40.2 %	5.12	0.53	MinTemp (1.0), Mgmt (0.47), Precipitation (0.44), Oxisols (0.39), MeanTemp (0.29), GrowTemp (0.28)
Asia	33.5 %	5.44	0.78	Precipitation (1.0), GrowTemp (0.65), Phenology (0.31), MaxTemp (0.25), Management (0.24)

We compared the performance of a binary RF classification problem to predict the region of natural and plantation tropical forests. Prediction error for management was lowest for natural forests and prediction error for region was lowest in the Americas. The most important variables varied by region. Temperature, leaf phenology and Entisol soils were determinative in the Americas (Fig. 4-6). In Asia, elevation and annual grow temperature were the primary drivers accounting for 23% of the variability in tNpp (Fig. 4-7). There was a moderate but significant negative correlation

between tNpp and maximum annual temperature $r(38) = -0.44$, $p = 0.004$ for Asian plantations where a maximum annual temperature of 16 °C showed 30 Mg ha^{-1} yr^{-1} tNpp with a threshold at 30 °C. Above this temperature, plantation tNpp declined to 15 Mg ha^{-1} yr^{-1} to a maximum annual temperature of 42 °C. This represents a 50% reduction in productivity for a 14 °C annual maximum temperature increase.

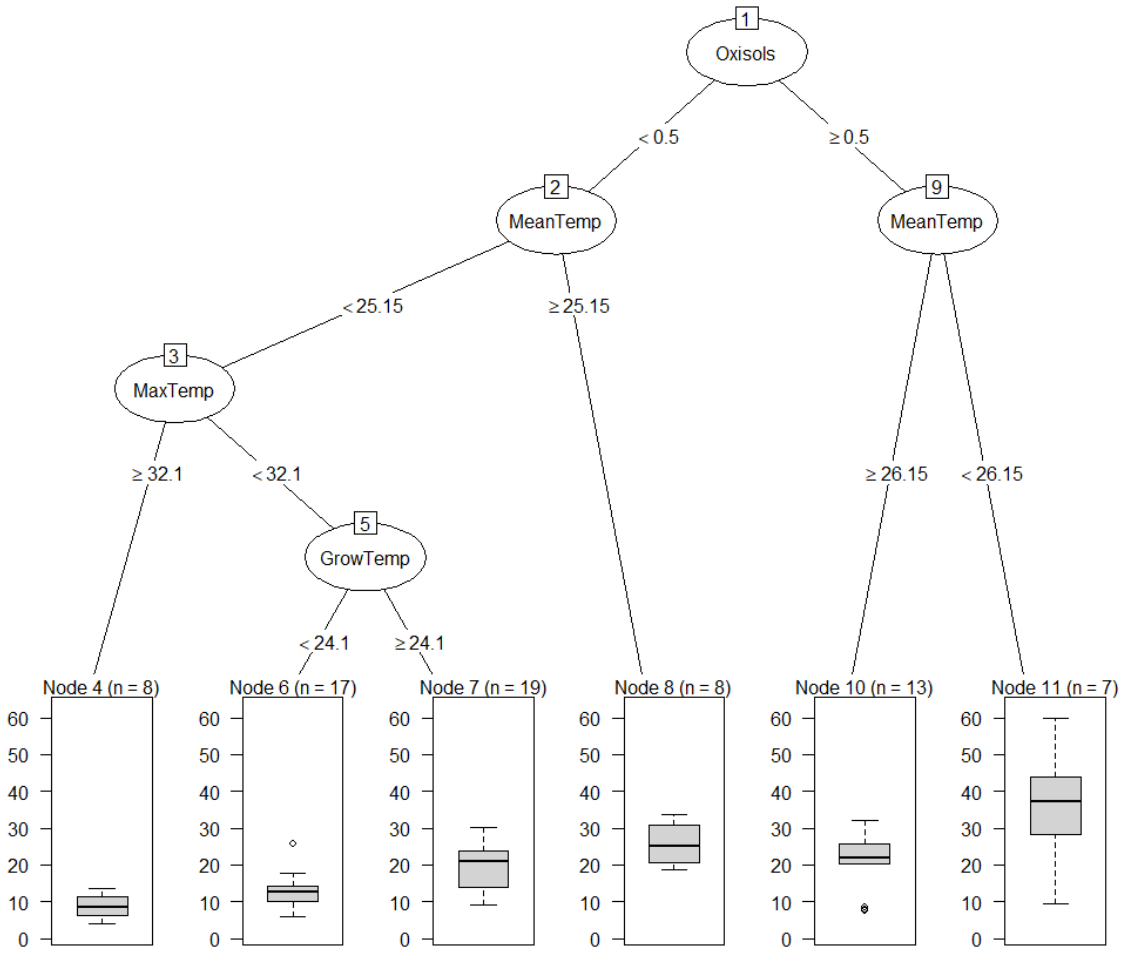


Figure 4-6: Random forest regression predictions for tNpp among natural forests and plantations in tropical climates of the Americas.

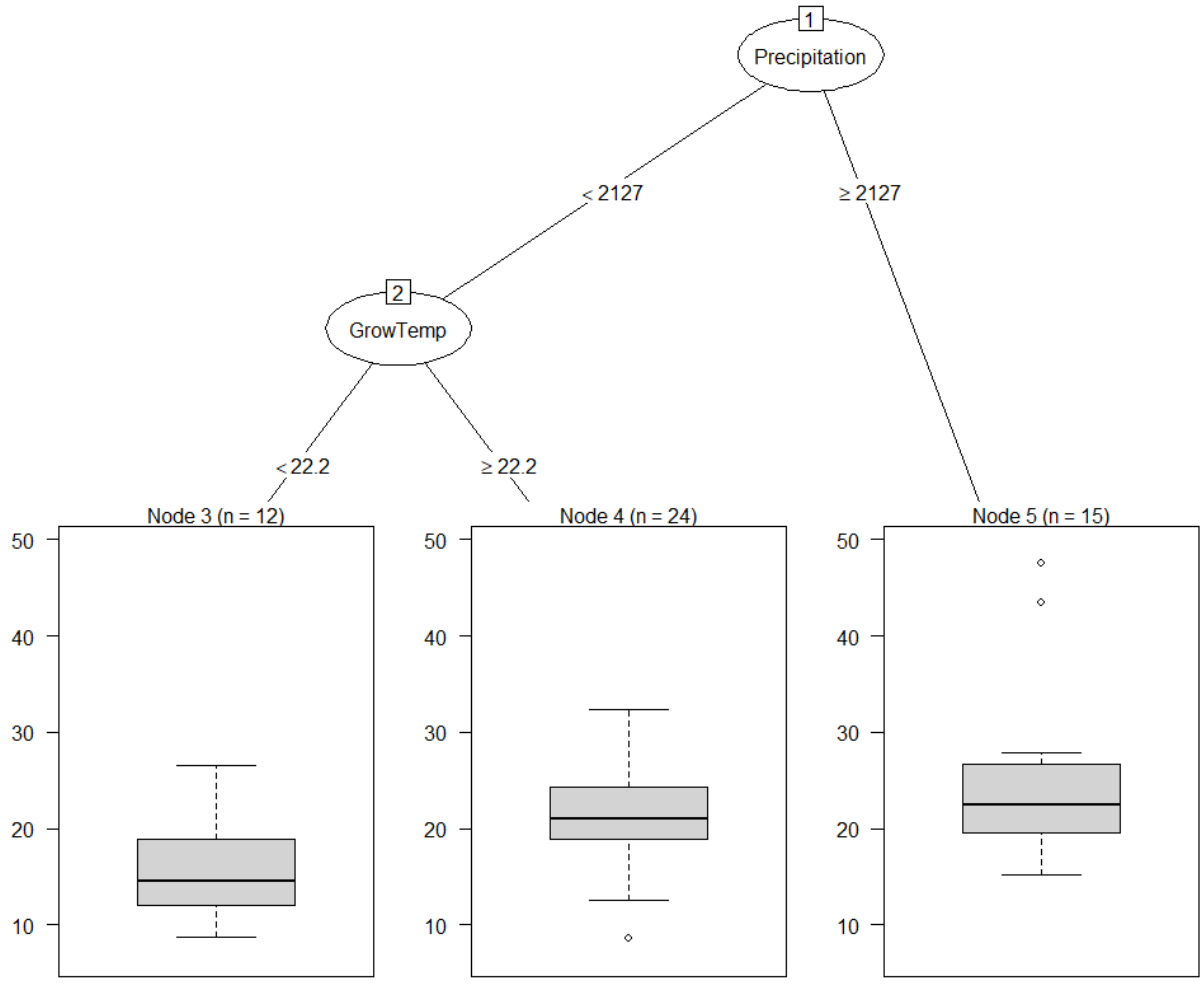


Figure 4-7: Random forest regression predictions for tNpp among natural forests and plantations in tropical climates of Asia.

3.5. Environmental productivity drivers varied by scale

To explore our hypothesis that tNpp and eFit were independent of phenological structure at the global-scale and more dependent on local-scale site conditions, multiple comparisons were made with Wilcoxon signed-rank tests for non-parametric and unbalanced data.

At the global scale, all phenological and climatic classifiers were removed. The first-order determinants of tNpp and eFit were climatic factors, minimum annual temperature, and precipitation. Validation of the tNpp model predictions gave coefficient of determination (R^2) = 0.83, RMSE = 3.9 Mg ha^{-1} yr^{-1}, MAPE (mean absolute percentage error regression loss) and MAE were equal to 27.5% and 4.1 Mg ha^{-1} yr^{-1}, respectively. Ecosystem fit model predictions (R^2) = 0.83, RMSE = 1.7 percent, MAPE (mean absolute percentage error regression loss) and MAE were equal to 26.5% and 1.9 percent, respectively (Fig. 4-8).

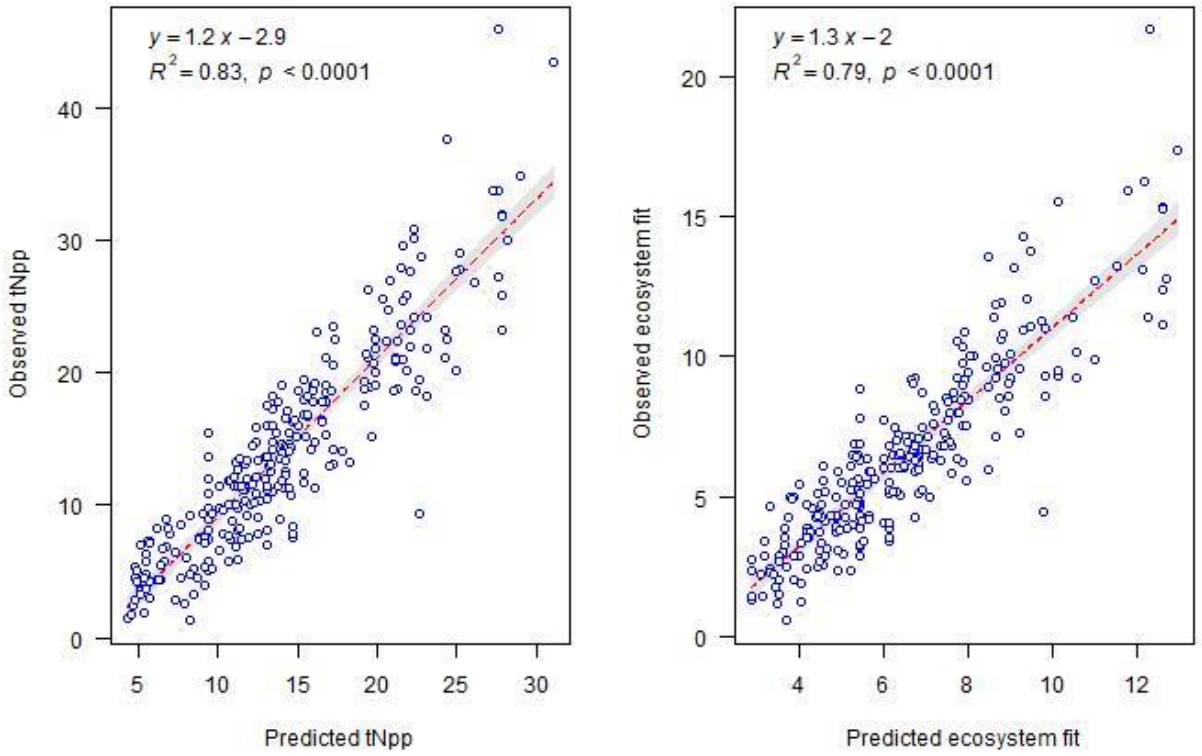


Figure 4-8: Model validation of field measures and random forest modeled tNpp and Ecosystem fit Random forest regression predictions for tNpp among natural forests and plantations in tropical climates of Asia.

3.6. Total net primary productivity (tNpp) and ecosystem fit (eFit)

Phenological leaf traits were not determinative of tNpp as each contrasting strategy was equivalent within each region. A global Kruskal-Wallis test indicated strong evidence of a difference between the mean ranks of tNpp for boreal and tropical climates (Fig. 4-9).

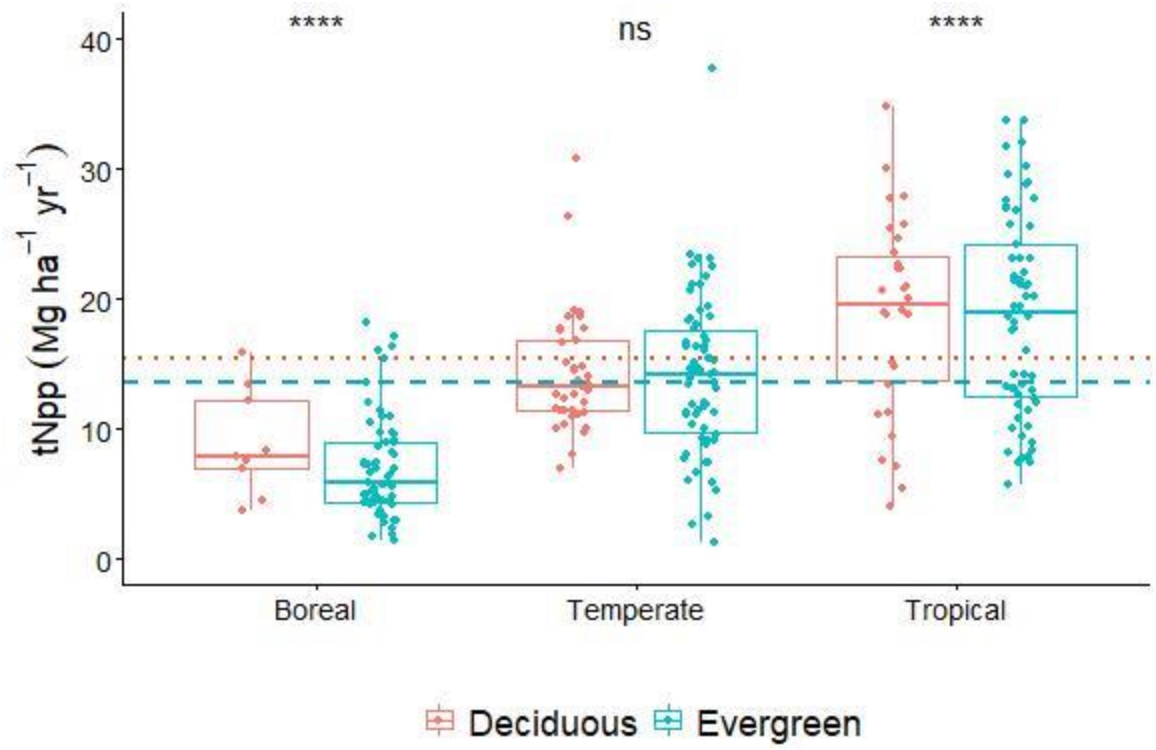


Figure 4-9: Summary of relationships between tNpp, climatic zone and leaf phenology for natural forests. Krustal-Wallis for climate (H(2), 102.8, p < 0.001) and phenology (H(1), 5.46, p = 0.02) indicated at least one significantly different climatic and phenological group. Significance level at top represents multiple pairwise tests against the base mean. Mean represented by a dotted horizontal line for deciduous forests and a dashed horizontal line for evergreen forests. Level of significance; * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$, **** = $p < 0.001$.

The difference in the range of eFit was narrow among all climates; boreal 13.0%, temperate 14.9%, and tropical 15.9%. In contrast to average tNpp, which increased in warmer climates, eFit was similar in all climatic zones. This indicates similar levels of local adaptation in all climates. There was a divergent trend by phenology in tropical and boreal climates. In tropical climates productivity was similar among deciduous and evergreens but evergreen trees generally had higher eFit than deciduous trees. In contrast, in the boreal zone, evergreens had lower productivity and lower eFit than deciduous trees. For tropical forests this trend could be due to evergreen trees not requiring additional resources for tissue growth when deciduous trees are competing for nutrients. In boreal climates seasonal leaf drop may confer benefits during winter months when free water is unavailable (Fig. 4-10). The temperate zone was not significantly different from the grand mean for Ec, tNpp or eFit which indicated latitudinal filtering at higher latitudes and towards equatorial regions.

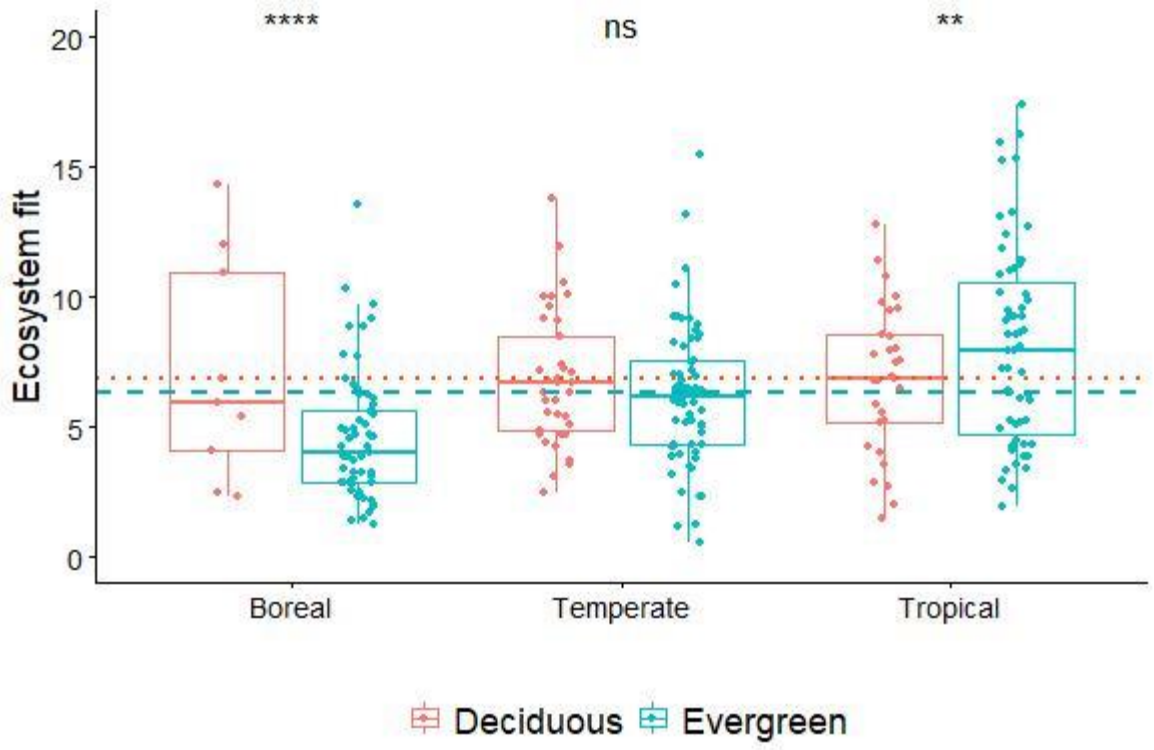


Figure 4-10: Summary of relationships between Ecosystem Fit, climatic zone and leaf phenology for natural forests. Krustal-Wallis for climate (H(2), 33.26, $p < 0.001$) and phenology (H(1), 3.72, $p = 0.05$) indicated at least one significantly different climatic and phenological group. Significance level at top represents multiple pairwise tests against the base mean. Mean represented by a dotted horizontal line for deciduous forests and a dashed horizontal line for evergreen forests. Level of significance; * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$, **** = $p < 0.001$.

3.7. Important variables distinguished tNpp by climatic zone

We hypothesized that the determinative variables for productivity and eFit would vary by climatic region due to interactions between climatic, environmental, and edaphic conditions. We anticipated that the relative importance of the variables would shift as the sensitivity of the response to site-level conditions varied region to region. A full multivariate RF regression model (n=267) for natural forests explained 65% of the variability. We identified climatic factors as first-order determinants of productivity, and we observed latitudinal environmental filtering effects on forest productivity. The effect of environmental filtering is known to shape forest community structure

and impact primary productivity but the role that site-level factors play in these patterns are less well understood (Shivaprakash et al., 2018).

Ecosystem fit and tNpp were invariant to phenological structure but were sensitive to site conditions and showed strong dependence to temperature, precipitation, elevation, Oxisol, Entisol, and Ultisol soils, and silty loam soil texture (Fig. 4-11). The tropical climate model explained the highest amount of variance and best predicted the response by the environmental variables. Although the tropical model performance was robust, it did suffer from outlier influence. In all climatic zones tNpp indicated higher prediction error than eFit (Table 4-6).

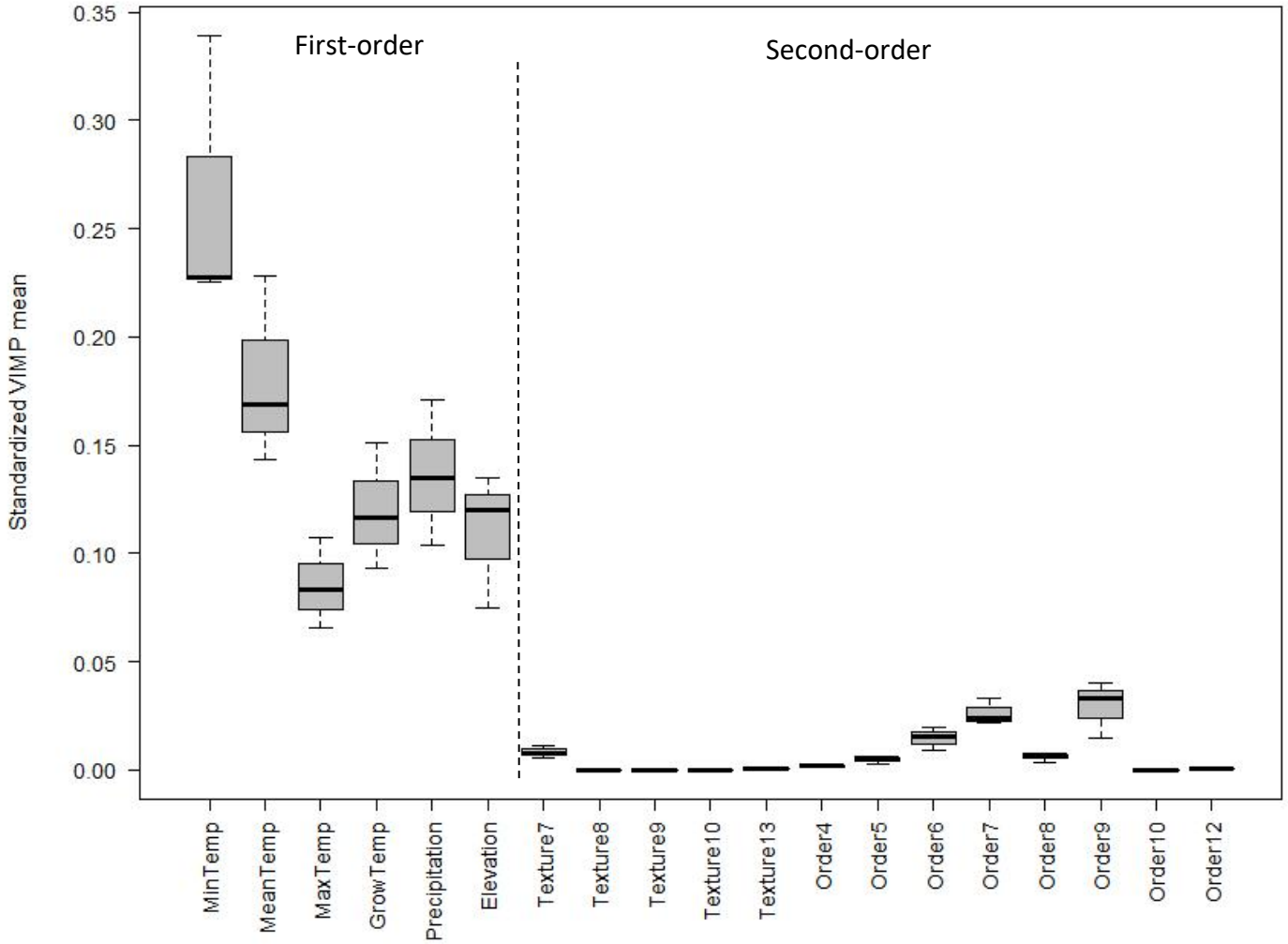


Figure 4-11: Standardized variable importance mean (VIMP) from the multivariate RandomForest regression model of tNpp and Ecosystem Fit (eFit) indicated climatic variables (temperature, precipitation), and elevation as first-order determinants and soil characteristics (soil order, soil texture) as second-order determinants for the most important splitting criteria for explaining productivity responses of natural forest ecosystems (n = 267).

Table 4-6: Model summary for predicting tNpp and Ecosystem Fit for each climatic zone. Pseudo R^2 is the percent variance explained by out-of-bag (OOB) predictions. Unexplained variance would be due to true random behavior or lack of fit. Out-of-bag error rate is the average error using predictions from the trees that do not contain their respective bootstrap sample. Standard error of the model (Std error) is the standardized error rates for response comparison within the same model. Model root mean square error (RMSE) is the tuned model error rate in units of the response and Model R^2 is the tuned model coefficient of determination Mean absolute error (MAE) is the error rate for the model robustness against outliers, and the coefficient of determination of the observed data regressed on the predictions from the trained model (Predictions R^2) represents overall model accuracy.

Model		Pseudo R^2	OOB Error Rate	Std Error	Model RMSE	Model R^2	MAE	Predictions R^2
Boreal forests: (n = 70)	tNpp	34.0	10.5	0.66	2.05	0.29	2.82	0.75
	Fit	41.7	4.78	0.59	1.36	0.32	1.80	0.78
Temperate forests: (n = 103)	tNpp	29.6	22.3	0.70	2.34	0.19	3.91	0.87
	Fit	31.0	4.81	0.69	1.06	0.18	1.83	0.88
Tropical forests: (n = 94)	tNpp	39.5	43.73	0.65	2.72	0.36	5.01	0.92
	Fit	44.8	8.49	0.59	1.17	0.42	2.08	0.92

The most productive systems were tropical forest growing in moderate moist conditions on Inceptisol soils (mean = 34 Mg ha^{-1} yr^{-1}) and Entisol soils (mean = 43 Mg ha^{-1} yr^{-1}) in Africa and Asia, respectively. The highest eFit was also in these types of tropical forests with a minimum temperature above 20 °C and precipitation greater than 2400 mm/year.

The regression tree visualization for tNpp indicated the first major split was minimum annual temperature with a threshold of 3.3 °C. The most productive forests to the right of the first split are characterized by Oxisol soils with sandy texture or a minimum annual temperature above 18.3 °C. In Figure 4.12, the first split to the left characterizes the lowest average forest productivity with mean annual temperatures less than 6 °C. Above 6 °C productivity is moderate and determined by interactions between maximum annual temperature and precipitation. Moderate productivity is also characterized by minimum annual temperatures above 3.3 °C and Entisol soils.

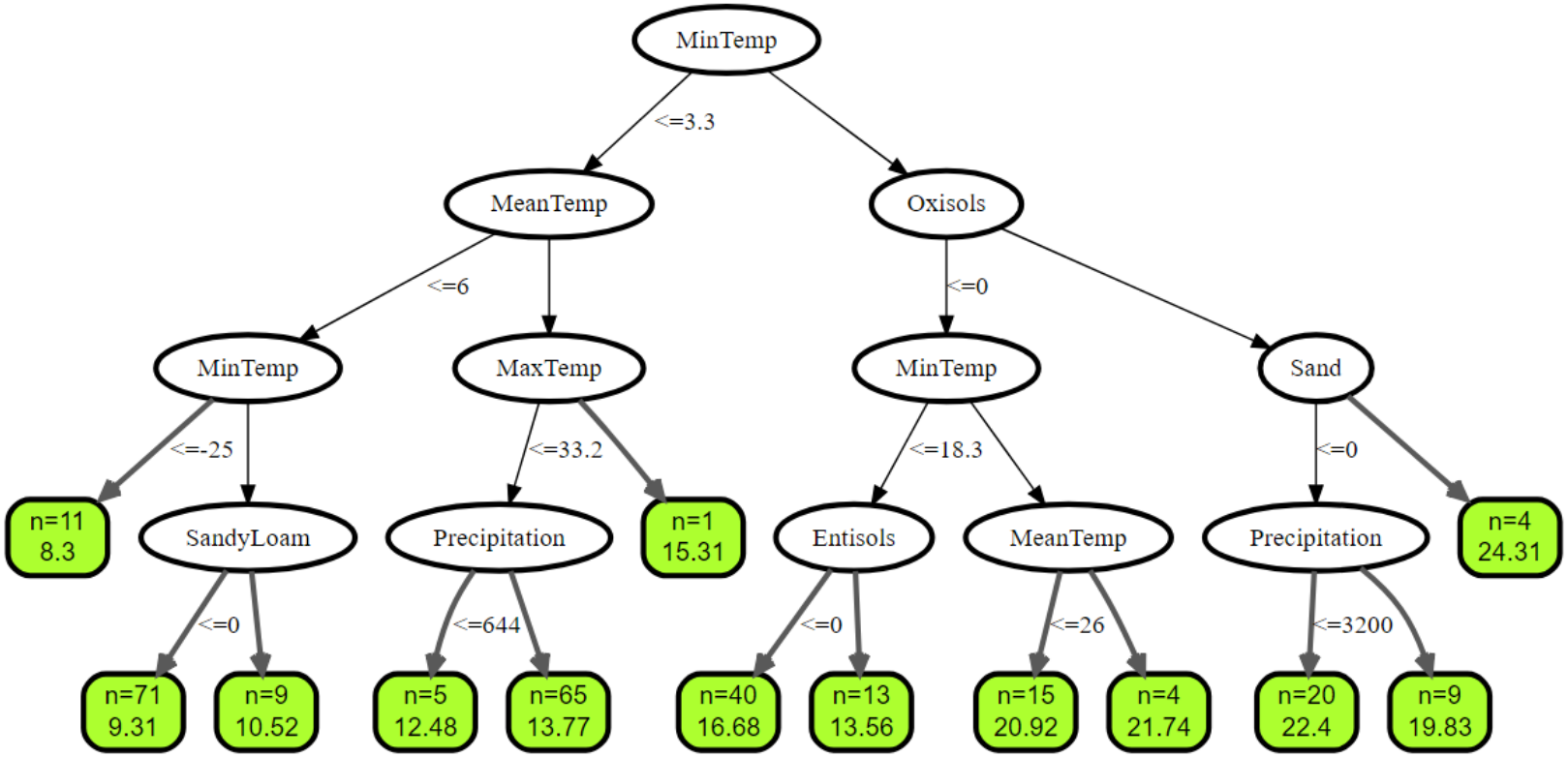


Figure 4-12: The binary random forest regression tree highlights the significant splits for explaining tNpp for 267 natural forests. These splits show the thresholds of climatic and edaphic variables. This regression analysis shows how it is a combination of variables that need to be used to explain productivity. Green boxes show number of forest sites and mean tNpp for that leaf.

Ecosystem fit was highly dependent on site-level soil conditions which indicated smaller-scale sensitivity than tNpp. The lowest percentages of eFit were found when mean annual temperature was less than a threshold of 3.8 °C. Moderate eFit percentages were characterized by having mean annual temperature above 3.8 °C and Entisol soils. The highest levels of eFit were bracketed by a mean annual temperature above 26.2 °C and a maximum annual temperature below 35 °C. A sandy-loam soil texture implied an average of 10.7% eFit (Fig. 4-13).

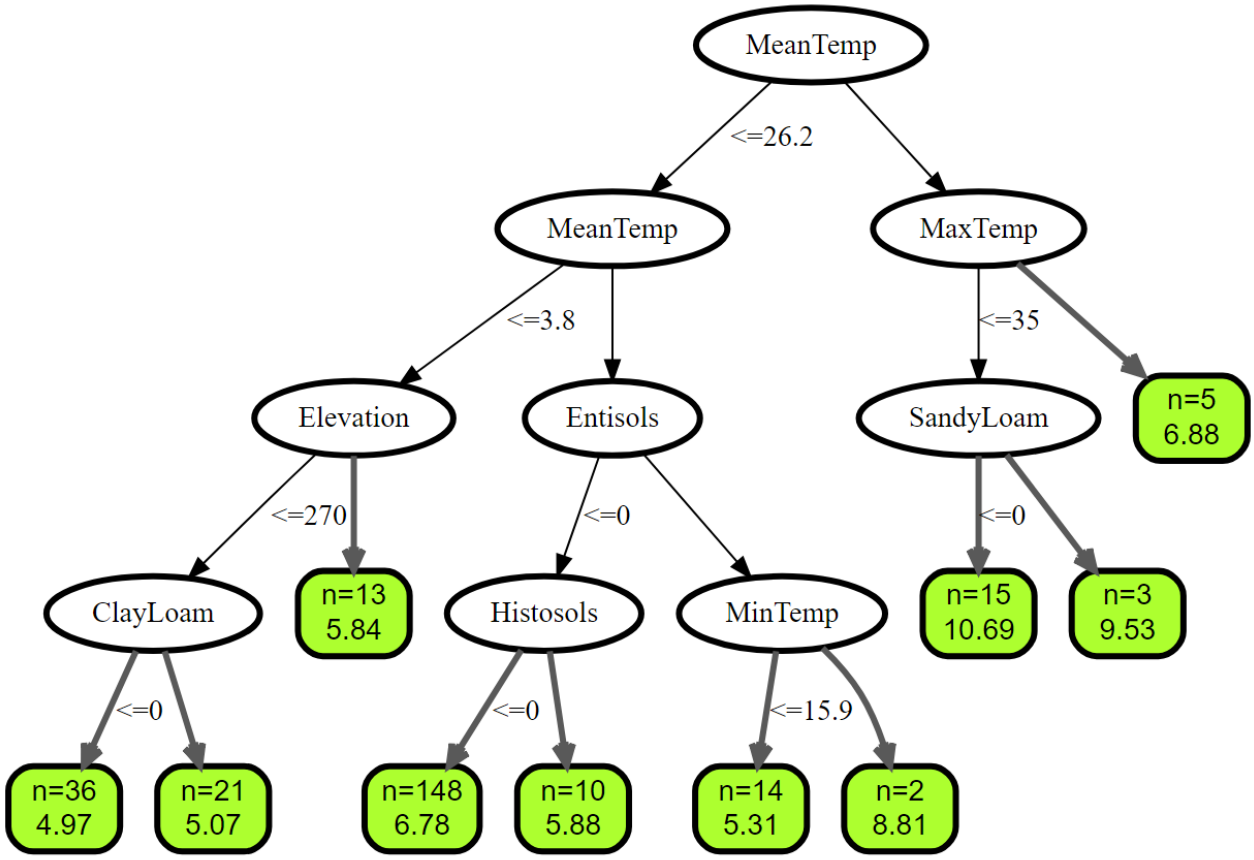


Figure 4-13: The binary RandomForest regression tree prediction model for Ecosystem Fit (eFit) representing natural forests. eFit is characterized by the interaction between temperature and soil conditions indicating a stronger site-level dependence. Zero represents condition not present.

These two regression trees demonstrate that tNpp and eFit although related, respond to different scales and different environmental conditions. Although similar productivity levels sometimes overlap (i.e., share different primary node splits) there is much more redundancy exhibited by eFit. Ecosystem fit percentage differences exhibited less than 2% overlap in all terminal branches except for forests constrained by mean and maximum annual temperature thresholds.

The RF prediction model for climatic zones representing natural forests indicated splits at thresholds of grow temperature, mean annual temperature and elevation accurately predict three primary forest zones with an error rate < 3%. A threshold of mean annual grow temperature of 15.6 °C is the first major node whereafter mean annual temperature of 4.1 °C and an elevation of 780 m split the remaining forests. At annual grow temperatures > 15.6 °C the model accurately predicted all tropical forests. At low grow and mean annual temperature and elevation above 780 m the model predicted boreal forests with a single error (Fig. 4-14).

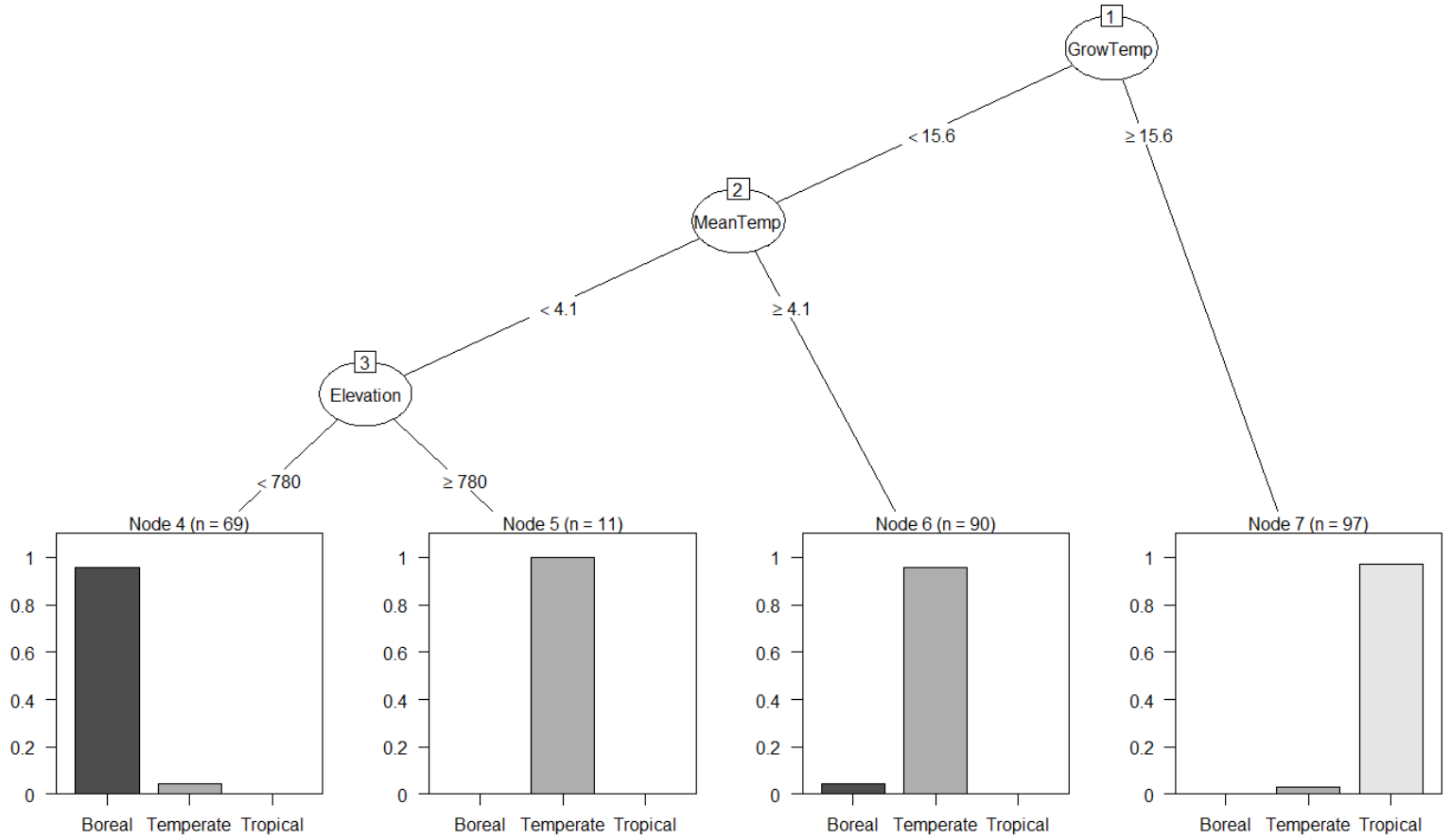


Figure 4-14: The RandomForest prediction model for climatic zones representing natural forests. These splits show the thresholds of grow temperature, mean annual temperature and elevation can accurately predict three primary forest zones with an error rate < 3 %. A threshold of mean annual grow temperature of 15.6 ˚C is the first major node whereafter mean annual temperature of 4.1 ˚C and an elevation of 780 m split the remaining forests. At annual grow temperatures > 15.6 ˚C the model accurately prediction all tropical forests. At low grow and mean annual temperature and elevation above 780 m the model predicted boreal forests with a single error.

4. Discussion

There has been a significant amount of interest in modeling and managing forests to mitigate climate change impacts. One response to this research has been to plant more trees (Popkin, 2019) without considering that the geographic distribution may be mismatched from its ecological niches (Pagel et al., 2020) or that the tree species planted do not support native species or biodiversity (Sigurdardottir, 1999). Understanding this mismatch between ecological and geographic niche is important because it begins to address the resilience of vegetative communities to specific environmental conditions or what is the range of adaptive responses available to a species in their ecological niche. It begins to highlight the importance of site scale factors on determining the resilience of a vegetative community to its environment when they grow outside of their ecological niche. This is like understanding that there is a narrow human climate suitability niche (Xu et al., 2020) but human survival is not limited to this narrow temperature band. Xu et al. (2020) reported a narrow climate band (~11 °C to 15 °C with a secondary mode at ~20 °C to 25 °C) - called the human climate niche - where high human population densities coincide with high crop yields and livestock production. In their most suitable temperature niche, humans still used technological advancements to reduce the constraints that limited their yields of crop yields despite this being an ideal environment for their survival. As Xu et al. (2020) wrote people migrating from their lands are probably linked to a critical climate threshold when they are unable to survive climatic events. Research in Indonesia reported how a temperature threshold above 25 °C determined when people would relocate from their homes and other disturbances, e.g., volcanic eruptions, but always returned to their homes (Wogan, 2014). Fagan (2008) in his book "The Great Warming: Climate Change and the Rise and Fall of Civilizations" wrote how the warmer temperatures during the

Medieval Period (CE 800 – 1200) resulted in the narrow temperature band conducive for cities to be built in northern Europe.

This human climate suitability band identified by Xu et al. (2020), coincides with the region that has been most altered by humans. In this suitability band it was easy to maximize vegetative growth and harvest natural resources, or to farm the lands. For example, 82% of temperate broadleaved forests have been altered by humans (Hannah et al., 1995) and today only 3% of the world's frontier forests are found in the temperate zone (Bryant et al., 1997). Historically the boreal and tropical forests were less populated by humans and less suitable for agriculture which explains why 70% of the world's remaining frontier forests are found in Russia, Canada, and Brazil (Bryant et al., 1997). These regions of the world identified by Xu et al. (2020) to be outside of this human climate niche, were historically less altered by humans are now starting to lose vegetation cover (Song et al. 2018). Their data also showed that many people survive and adapt to their environments outside of these narrow temperature bands where agricultural production is limited by old and highly weathered soils, or where they receive too much or too little rainfall. In this case, it is not humans who are adapting to their environment, but their success to surviving in those bands due to their ability to harvest resources from forests which are adapted to a heterogeneity of environmental conditions. In 1997, 48% of the world's frontier forests were in the boreal biome and 44% in the tropical climatic zone and these are the same regions that today are important sources of global lumber and paper supplies. Research has reported how 60% of the changes in global land cover (between 1982 – 2016) are directly due to human activities in a human-dominated Earth system (Song et al., 2018). Decisions on which trees to plant and how to manage land-use practices to minimize or reduce their impacts needs to tailor site specific practices based on an understanding of how they impact the resilience and productive capacity of a forest.

The ability to identify a theoretical maximum potential productivity, i.e., reference tNpp, (Gordon et al., 1992), and identify the critical thresholds of productivity can help to determine when tipping points in complex ecosystems may occur by identifying empirical indicators that a system or components of the system may shift to another state (Carpenter et al., 2011; Scheffer et al., 2012). This type of information is more valuable than knowledge of a mean tNpp or biomass level at the biome or landscape level which identifies climatic indicators as the primary controllers of productivity. To understand how forest production and biomass may change in response to climate change or to land-use, the resilience of each system needs to be known to identify when ecological regime changes may occur. It would help climate modelers to know when a change in climate, modified by its edaphic of soil context, will reduce the resilience of a forest to further disturbances, such as drought.

4.1. Global tNpp Estimates ≠ Site Level tNpp and Ecosystem Fit

In 1998 it was proposed that a ratio of total net primary productivity (tNpp) to gross primary productivity (GPP) of ~0.47 ± 0.04 could be used to simplify modeling of forest productivity but stressed the importance of better understanding of environmental factors impacting carbon shifts between the above- and belowground (Waring et al., 1998). In 2020 these authors reiterated that comparison of forest productivity across large spatial and temporal scales should be founded on relatively basic relationships (Landsberg et al., 2020) after Collalti & Prentice (2019) questioned the use of a universal NPP/GPP ratio of 0.47±0.04 to derive NPP using models. Landsberg et al. (2020) responded to Collalti & Prentice (2019) that the NPP/GPP ratio varied from 0.22 to 0.79. They further state that the ability to quantify the variation of carbon flux to and from soils in different forest ecosystems with varying environmental conditions could be used to explain the reasons for the variations which would foster the development of simplified productivity models at

the scale of individual forest stands (Landsberg et al., 2020). In this study, we take a more basic mechanistic approach by bridging basic photosynthetic physiological parameters, from a well-established body of knowledge, with a theoretical productivity potential and empirical data obtained directly on site. This variance in the NPP/GPP ratio is also why there is a need to develop a different reference condition to estimate the theoretical potential productivity at each site that does not vary based on the diversity of adaptive mechanisms of trees to their edaphic and climatic environments. This research compared forest ecosystem properties across climatic regions to estimated thresholds of environmental conditions, tree phenology and an index of eFit to estimate the vulnerability of different forest ecosystems to climate change (Table 4-7).

Table 4-7: Summary of marginal partial dependence of the most important environmental variables from RF regression model of natural forests.

	tNpp				Ecosystem fit			
Climate	Variable	IncNode Purity	Partial dependence	Safe operating space (SOS)	Variable	IncNode Purity	Partial dependence	Safe operating space (SOS)
Boreal (n = 70)	Precipitation	137.4	-	< 750 mm	Elevation	102.7	+	0 - 750 m
	Maximum temp	129.8	+	> 16 ˚C	Precipitation	85.6	nm	< 750 mm
	Grow temp	117.3	nm	5 - 12 ˚C	Grow temp	61.2	nm	5 - 12 ˚C
	Elevation	107.1	+	0 - 750 m	Min temp	54.1	+	> -8 ˚C
	Minimum temp	104.8	+	-37 - 0	Max temp	40.9	nm	7 - 28 ˚C
	Mean temp	65.7	w +	-10 - 15	Mean temp	34.8	hs	-5 - 14 ˚C
	Soil texture	54.2	+	Clay-loam* (n=30%)	Soil order	18.8	+	Andisol*(n=4%)
Temperate (n = 103)	Precipitation	571.8	+	< 1000 mm	Elevation	118.4	-	0 - 3150 m
	Min temp	471.2	+	> 0 ˚C	Precipitation	105.0	hs	500 - 3500 mm
	Elevation	379.3	nm	0 - 3150 m	Min temp	87.0	nm	> -20 ˚C
	Max temp	341.0	-	> 25 ˚C	Max temp	76.7	-	> 25 ˚C
	Mean temp	328.8	nm	0 - 25 ˚C	Mean temp	76.1	-	0 - 25 ˚C
	Grow temp	260.5	hs	7 - 17 ˚C	Grow temp	56.2	w -	7 - 17 ˚C
	Soil order	124.0	+	Ultisol (n=10%)	Soil order	20.1	+	Ultisol (n=8%)
	Soil texture	60.0	-	11% Silt-loam	Soil order	15.8	+	Inceptisol (n=38%)
Tropical (n = 94)	Min temp	889.1	nm	-6 - 32 ˚C	Min temp	204.6	nm	-6 - 32 ˚C
	Precipitation	836.3	nm	700 - 7670 mm	Precipitation	173.9	+(-)	2000 mm
	Mean temp	812.9	+	9 - 29 ˚C	Mean temp	169.6	+	> 20 ˚C
	Elevation	756.9	hs	0 - 3060 m	Grow temp	160.9	+	> 25 ˚C
	Grow temp	736.8	+	> 24 ˚C	Elevation	150.1	hs	0 - 3060 m
	Max temp	546.6	+	10 - 42 ˚C	Max temp	98.9	+	10 - 25 ˚C
	Soil order	202.9	-	Entisol (n = 19%)	Soil texture	39.0	+	Sandy (n = 13%)
	Soil texture	201.9	+	Sandy (n = 13%)	Soil order	30.6	+	Oxisol (n = 19%)

i) Increase in node purity: measure of relative variable importance = decrease in the residual sum of squares (RSS)/variance in the node, i.e., the weighted average of incremental purity reported at each split by the variable used for the split, with the node population as the weight.

ii) Partial dependence: change in response to change in the variable when all other variables held at their mean. Characterization of the curve denoted as; “hs” = hard saturation represents no dependence within a range of the variable for the climate, “+” = positive monotonic dependence, “-” = negative monotonic dependence, nm = nonmonotonic dependence or unusual curves. A weak response is “w.”

iii) The safe operating space (SOS) is the range of the variable where the phenotypic response shows dependence. Any range of the variables where the response shows resistance to the variable is considered outside the SOS. Soil percentage and name represent the proportion of sites with the soil condition that produced the highest response value.

To understand the impact of climate change on a local forest ecosystem, researchers need to identify: (1) a reference upper threshold of productivity for each forest stand specific to the site and calculated using variables external to the site so it is not influenced by variable site-specific factors, and (2) the Safe Operating Space (SOS) where forests adapt to site constraints and disturbances. The reference theoretical maximum net primary productivity or potential NPP (TNpp) should be derived using external site factors or it will be challenging to determine its "Ecosystem Fit," (tNPP/TNpp) (Gordon et al., 1992). The reference TNpp expresses how well a forest is adapted to its site, i.e., its fit, and does it have capacity to grow more biomass beyond its current growth rate. The diversity of soil conditions coupled with climate, stand age, successional stage all influenced fine root dynamics in boreal forests (Yuan & Chen, 2010) which is the heterogeneity of environmental conditions that influence growth. All these factors will decrease the potential growth rate reached at a site (Vogt et al., 1996). As this research has shown, these internal ecosystem factors result in various combinations of constraints that will determine total productivity and how much carbon is allocated to roots. To compare sites will require the development of an external productivity measure that defines the maximum potential productivity for that site. For forest stand level assessments, an internal reference gross carbon flux would vary in an unpredictable manner and would reflect the variable influence of site-specific factors on productive capacity.

Ecosystem Fit can be used to assess how well a forest is adapted to its site constraints to grow biomass because it uses a reference maximum productivity level that is calculated with factors that are external to the site and not influenced by internal site conditions. The most important climatic and edaphic variables can be used to identify the "safe operating space" at each site, where the dependence of eFit is strongest within the upper and lower bounds of tolerable environmental conditions. Ecosystem Fit describes the percent of the theoretical Productive capacity that is realistically reached at a site because of site level conditions. The importance of site constraints in

limiting annual growth is seen in the fact that forests only grow up to 25% of the theoretical growth that is possible on the site. The theoretically productive capacity describes the reference productivity for each site. Ecosystem fit can be used as a tool to identify which forests are better adapted to their current growth environment and could be used to indicate which forests could be more vulnerable to climate instability and in need of management intervention.

Researchers have known for decades that as one moves from the local scale to larger landscapes that the amount of information decreases that is needed to predict processes occurring at larger landscapes and that climatic factors become most important predictors (Gosz, 1992; Vogt et al., 1997). Hierarchy theory emerged when the need to acknowledge the existence of different levels of biological organization were recognized (O'Neill et al., 1986). Models predicting NPP are sensitive to spatial sampling resolution and sampling selection criteria which explain the variance in the prediction (Gmur et al., 2014). Gmur et al. (2014) used the statistical method "`randomForest`" (Liaw & Wiener, 2002) to identify at each scale the significant variables in the predictive models. This study used five cell sizes, with the smallest cell size used being 1 km and which is the size also used by the MOD17A2/A3), to estimate GPP and tNpp (Running & Zhao, 2015). In Indonesia, minimum daytime temperature was the most important variable for the 20, 15, and 10 km grid-cell sizes but decreased in importance to becoming the third and tenth most important variable for the one and 5 km grid-cell size. Besides temperature variables, other variables affecting tNpp were elevation, fraction of absorbed photosynthetically active radiation and leaf area index but edaphic factors were not identified as being important (Gmur et al., 2014).

In this study, the varying thresholds of tNpp were produced by climatic factors when analyzing data by the boreal, temperate, and tropical climatic zones. Boreal forests had moderate levels of productivity were wide-ranging throughout the ecosystem property "space," but eFit was tightly constrained. This indicates adaptation to a diversity of environmental conditions whereas the

most productive boreal forests were limited to a much narrower window of eFit "space." For the boreal climatic zones, the most important variables constraining growth and eFit were maximum annual temperature and precipitation. The interaction between maximum annual temperature and precipitation indicated the highest levels of tNpp would be found where maximum annual temperature did not exceed 20 °C and annual precipitation was less than 500 mm yr^{-1} but moderate productivity levels were predicted with all levels of precipitation at that temperature. There was additional high productivity among boreal forests where maximum annual temperature did not exceed 14 °C and precipitation was below 1500 mm yr^{-1} (Fig. 4-15A). The highest eFit was expected at elevation above 600 m (Fig. 4-15B). There was moderate phenological and environmental interactions with maximum annual temperature. A steady increase of tNpp was noted with increasing maximum annual temperature above 16 °C. Divergence occurred as tNpp declined in deciduous forests an average of 2.5 Mg ha^{-1} yr^{-1} as precipitation approached 750 mm yr^{-1} (Fig. 4-16A). Evergreen forests were resistant to precipitation and productivity continued to improve through the measured range of the variable (Fig. 4-16B). With moderate increases in elevation eFit of deciduous forests increased by 7% indicating that eFit was depressed in boreal climates at elevations below 375 m whereas evergreen forests were resistant to climate changes at all elevations (Fig. 4-17A). There were contrasts between phenology with eFit and precipitation as evergreens improved a modest 1% and deciduous declined by 1%. This indicates rather weak directional selection based on phenology (Fig. 4-17B).

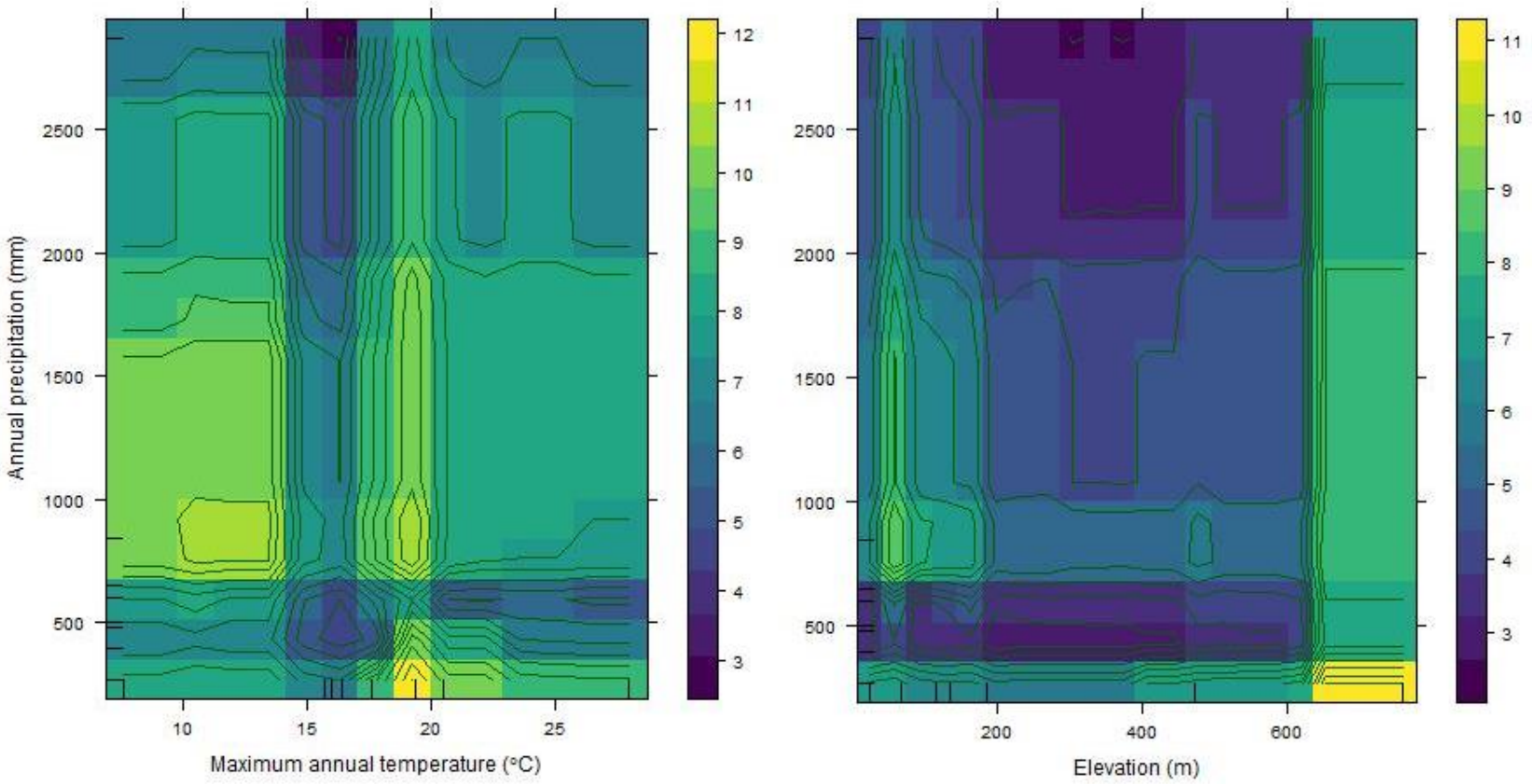


Figure 4-15: Partial dependence of tNpp and ecosystem fit in boreal forests Partial dependence plots for boreal forests. Contours represent the interaction between the two most important variables for (A) tNpp (Mg ha^{-1} yr^{-1}), and (B) Ecosystem Fit (%).

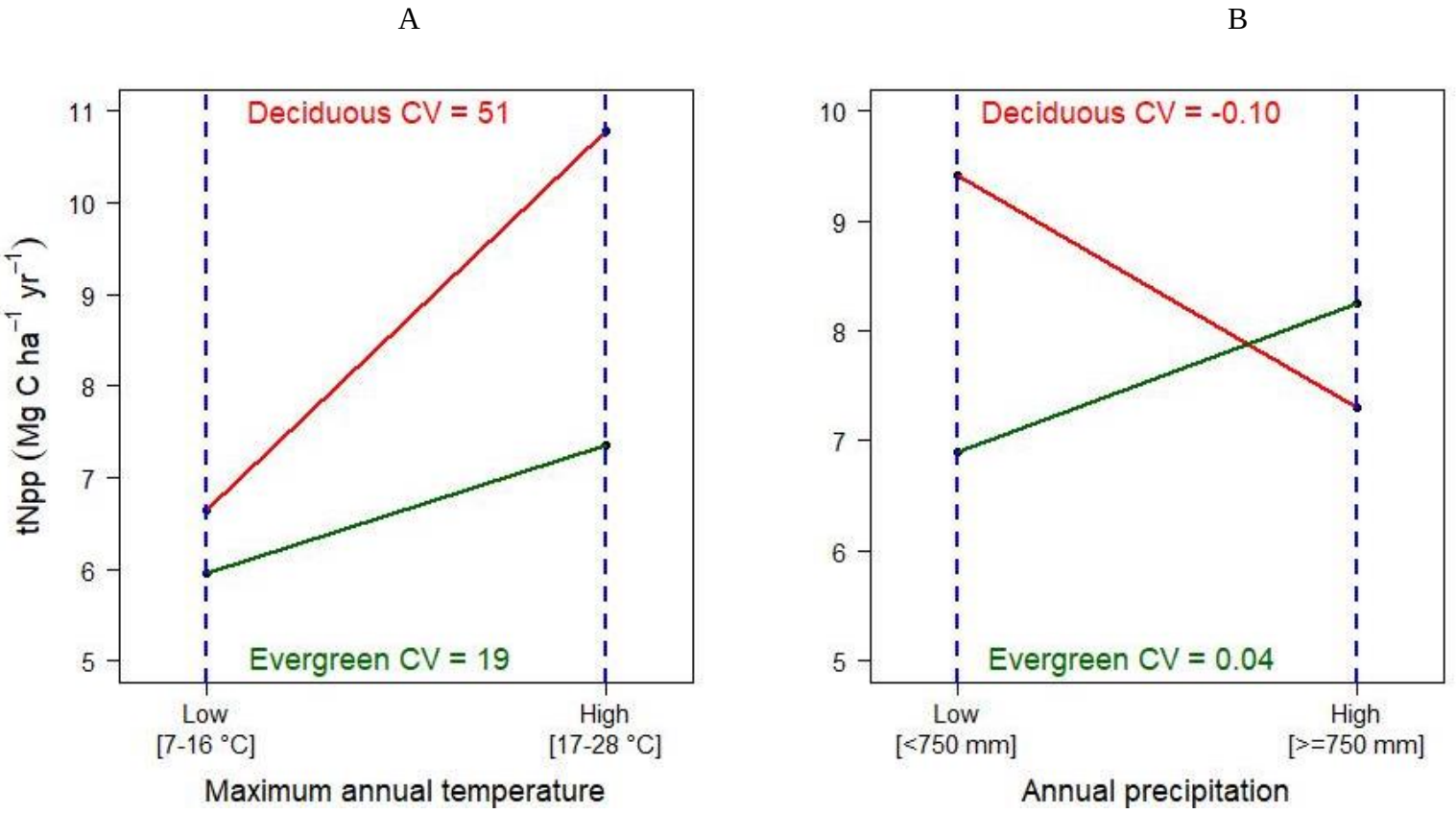


Figure 4-16: Norm of reaction plots for boreal forests. (A) Phenology drives diverging tNpp with maximum annual temperature. (B) Phenological traits interact with annual precipitation above 750 mm reaching tNpp saturation between 7-8 Mg ha^{-1} yr^{-1}.

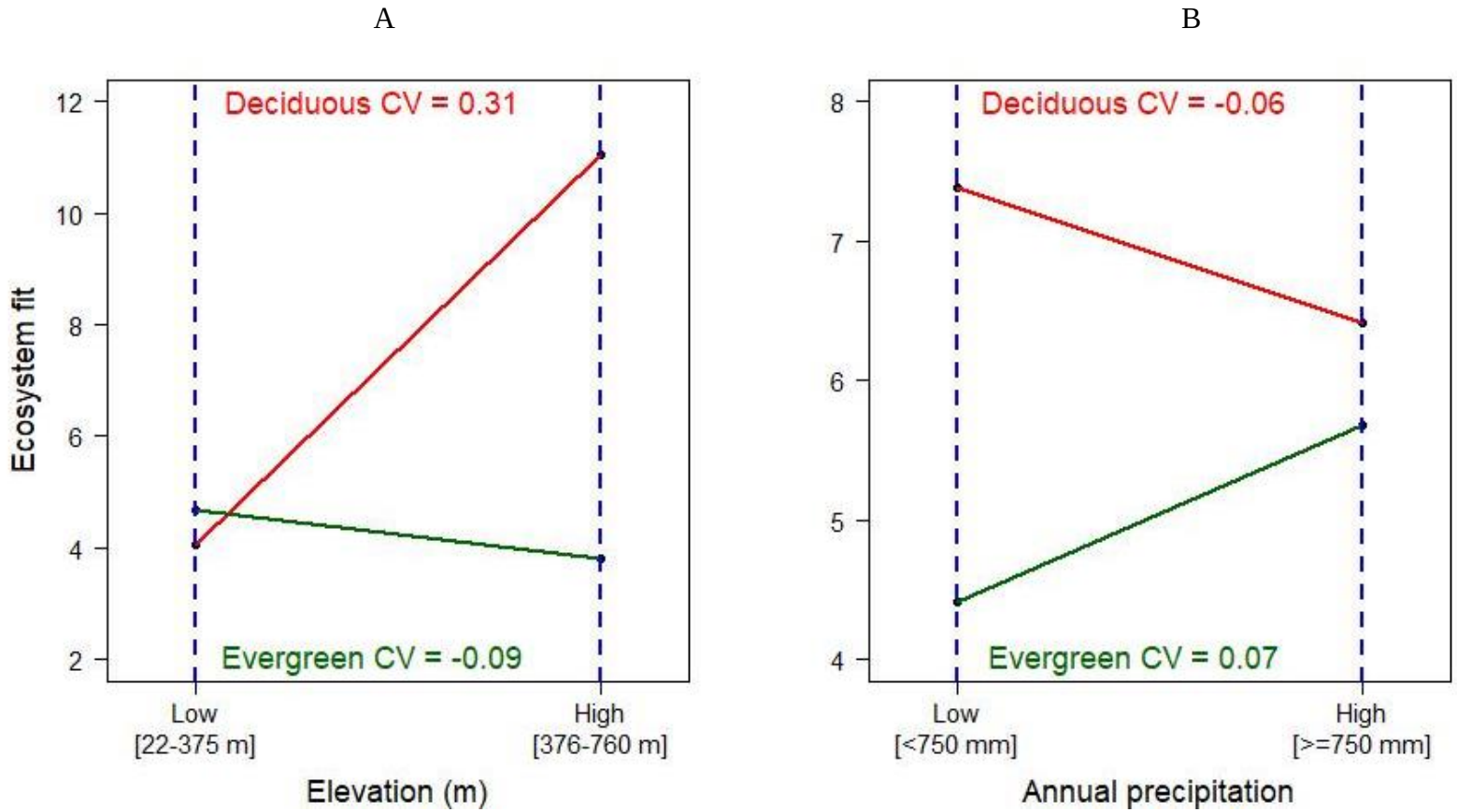


Figure 4-17: Norm of reaction plots for ecosystem fit in boreal forests Reaction norm plots for boreal forests. (a) Phenology shows an interaction for Ecosystem Fit (eFit) at low elevations. Above 375 m deciduous forests show steady increase in eFit as evergreen forests are stable at ~ 4% eFit. (b) Whereas eFit in evergreen forests shows an increase in annual precipitation, deciduous forests show an eFit decrease.

Similar to the boreal climate zones, forests growing in the temperate climatic zones had tNpp and eFit clearly segregated in ecosystem property space but limited data values moderately skewed the predictions. There were clear indications that minimum annual temperature, precipitation, and elevation were determinative of the productivity. The "productivity space" was most confidently limited to conditions where minimum annual temperatures exceeded 5 °C, elevations were below 500 m and precipitation levels were high. There was also a second moderately high level of eFit near 1000-meter elevation. All other conditions produced approximately 50% lower levels of growth (Fig. 4-18A). Ecosystem fit was wide-ranging and could be found over a variety of combinations of environmental conditions indicating higher local-level adaptation to maximize eFit. Ecosystem fit was severely compromised at elevations above 1000 m and precipitation below 200 mm yr^{-1} and again at median elevation and precipitation levels (Fig. 18B).

Interactions among temperate forest properties were strong as deciduous forest tNpp declined 3 Mg ha^{-1} yr^{-1} and evergreens increased approximately 2 Mg ha^{-1} yr^{-1} with precipitation >1000 mm yr^{-1} and when minimum annual temperature rose above 0 °C (Fig. 4-19A). Ecosystem fit showed weak dependence with elevation but a stronger interaction between phenology and annual precipitation as the eFit of deciduous forests declined by more than 3% when precipitation levels were over 1250 mm yr^{-1}. Evergreen forests were largely resistant to changes in elevation and precipitation level (Fig. 4-20A, Fig. 4-20B) and more resistant in temperate forests than other climates. This indicates rather weak directional selection based on phenology.

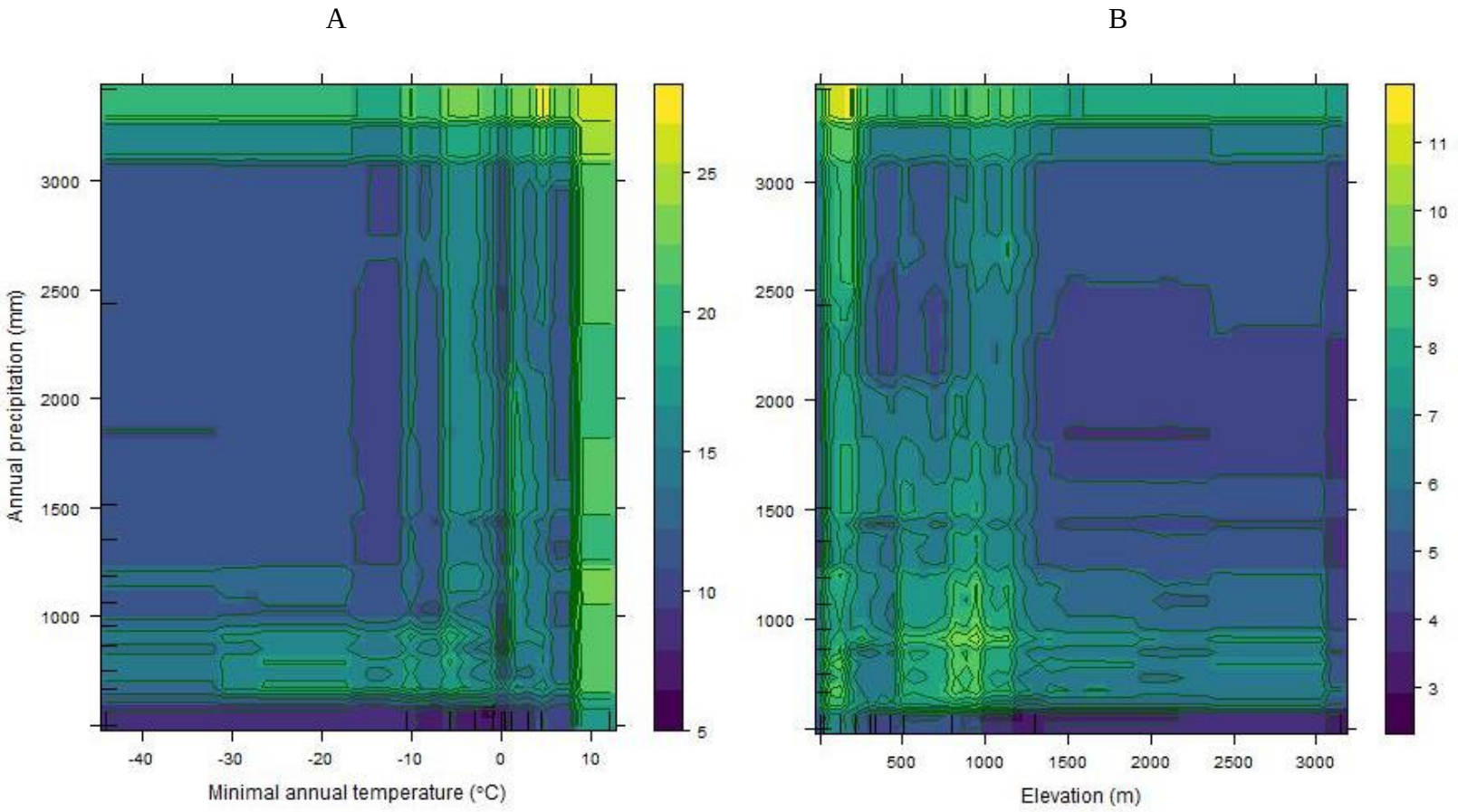


Figure 4-18: Partial dependence plots for temperate forests. Contours represent the interaction between the two most important variables explaining (a) tNpp (Mg ha^{-1} yr^{-1}) and (b) Ecosystem Fit (eFit) (%).

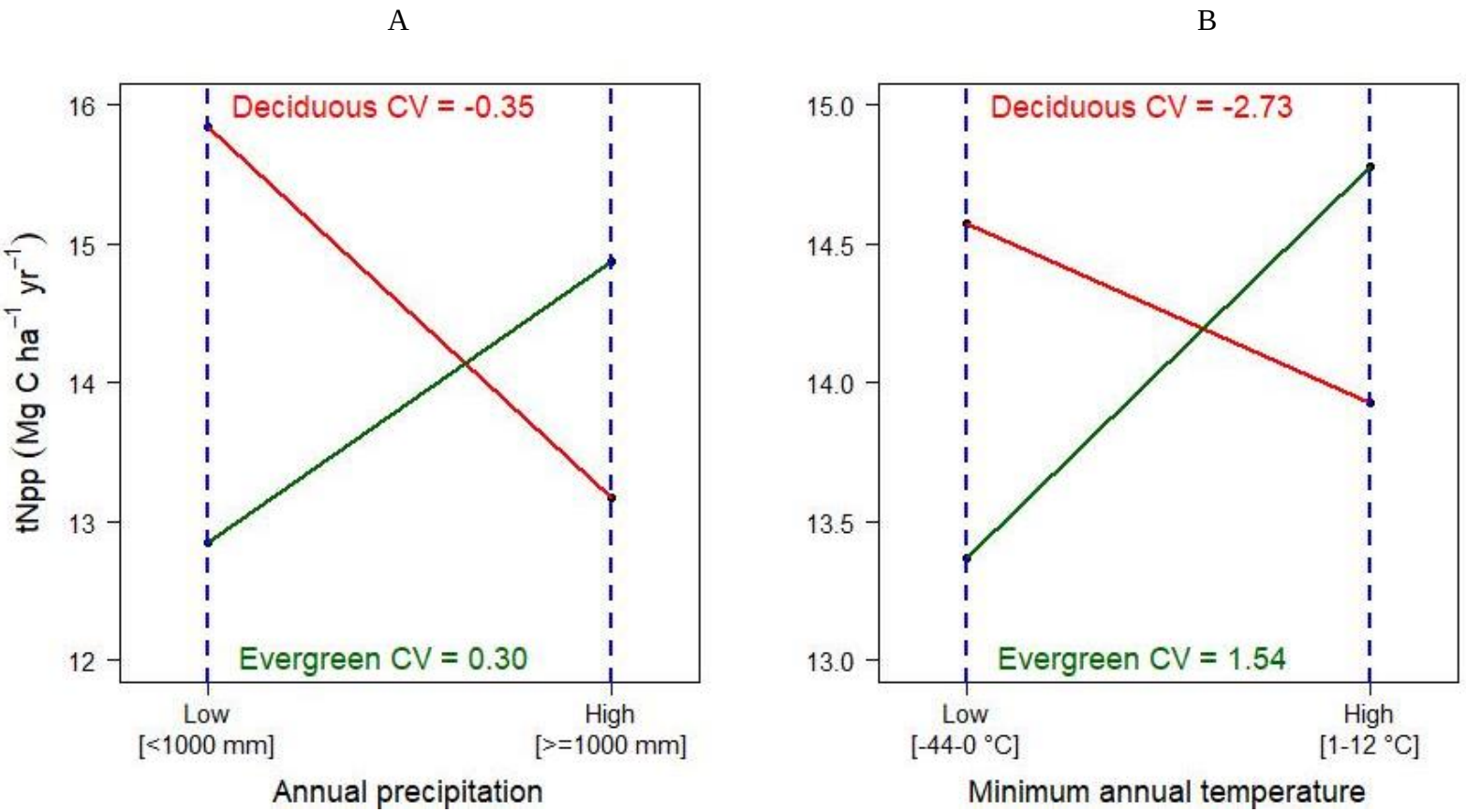


Figure 4-19: Norm of reaction plots for tNpp in temperate forests. Phenology shows an interaction with (A) annual precipitation and (B) minimum annual temperature. Productivity in deciduous forests decreases under high levels of precipitation and higher ranges of minimum annual temperature, as evergreen forests increase. The overall impact to tNpp is moderate, ranging from 1-3 Mg ha^{-1} yr^{-1}.

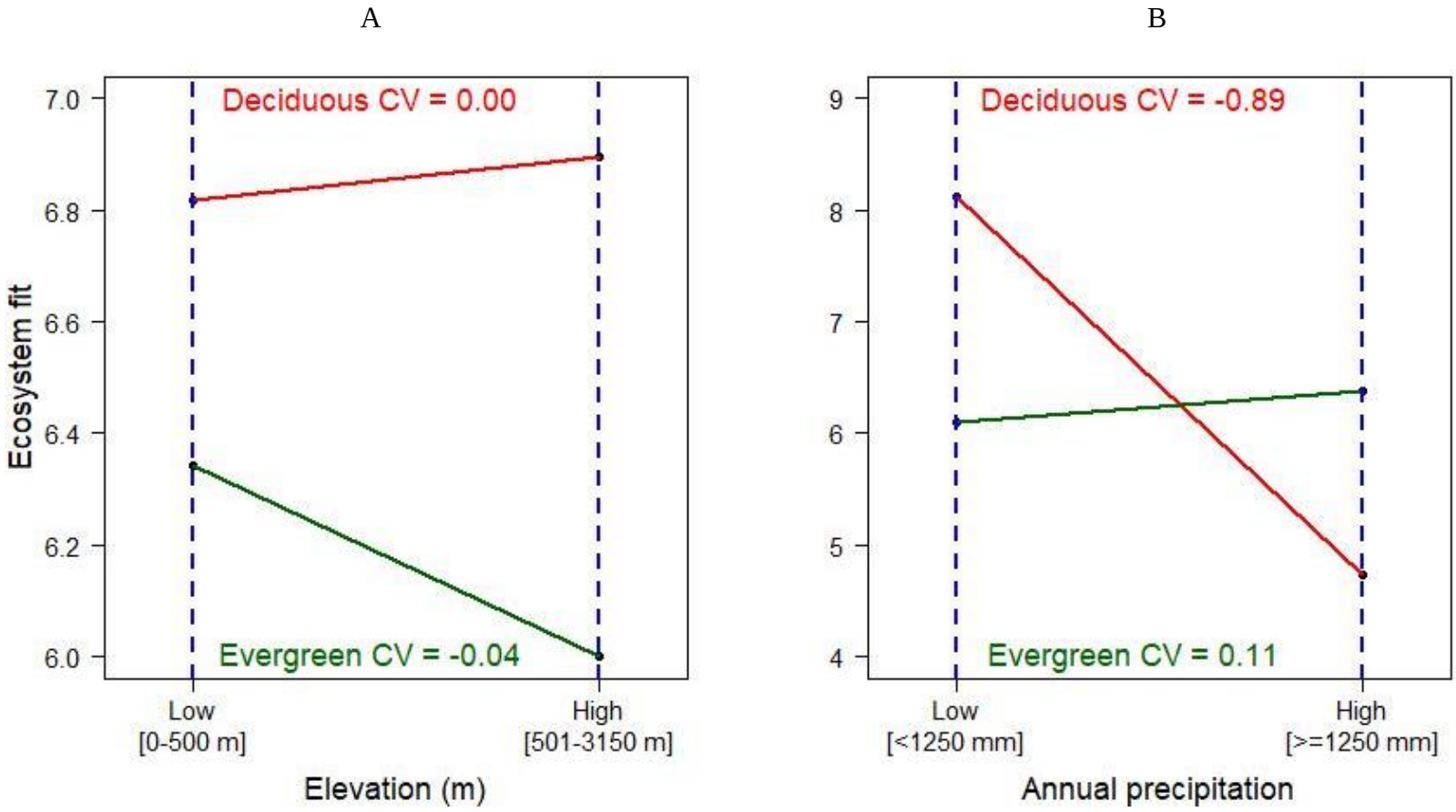


Figure 4-20: Reaction norm plots for temperate forest. (A) Evergreen phenology shows slight dependence of Ecosystem Fit (eFit) to increasing elevation. (B) Whereas deciduous forests show declines >3% with high levels of precipitation as evergreen forests are resistant.

Tropical forests exhibited more diversity across ecosystem property space compared to the boreal and temperate climate zones. This is indicative of wide-spread local adaptation, finely tuned niche partitioning and high biodiversity. Although the very highest productivity space (> 30 Mg ha^{-1} yr^{-1}) was limited to sites where precipitation averaged 2000 mm per year and minimum annual temperature exceeded 20 °C, moderate tNpp (20-30 Mg ha^{-1} yr^{-1}) were found across a wide-variety of environmental conditions. For example, forests with similar productive capacity were predicted where precipitation was only 1000 mm yr^{-1} but also where precipitation was greater than 6000 mm yr^{-1} (Fig. 4-21A). The eFit space behaved similarly, indicating that forest with high eFit would be scattered across a wide variety of interacting site conditions. The forests most constrained in eFit was predicted where very high precipitation rates (> 5000 mm yr^{-1}) overlapped with low minimum annual temperatures (< 5 °C) (Fig. 4-21B). The strongest interactions between phenology and the environment were evident in the tropics (Fig. 4-22A, Fig. 4-22B). Deciduous forest tNpp and eFit declined by 7 Mg ha^{-1} yr^{-1} and 2.5%, respectively, and then reversed and increased > 7 Mg ha^{-1} yr^{-1} and ~10% with high precipitation (Fig. 4-23A, Fig. 4-23B).

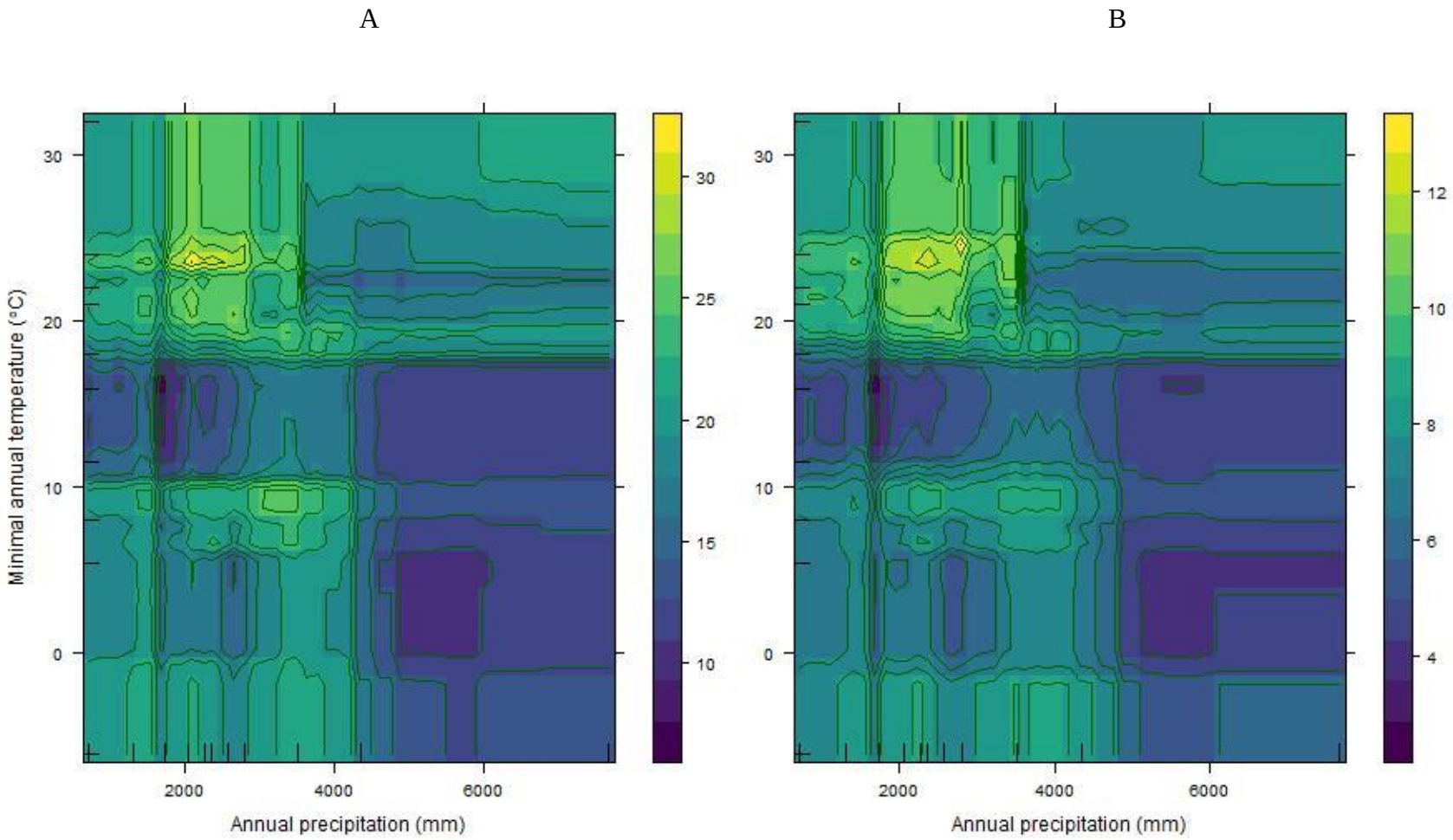


Figure 4-21: Partial dependence plots for tropical forests. Contours represent the interaction between the two most important variables explaining (A) tNpp (Mg ha^{-1} yr^{-1}) and (B) Ecosystem Fit (%).

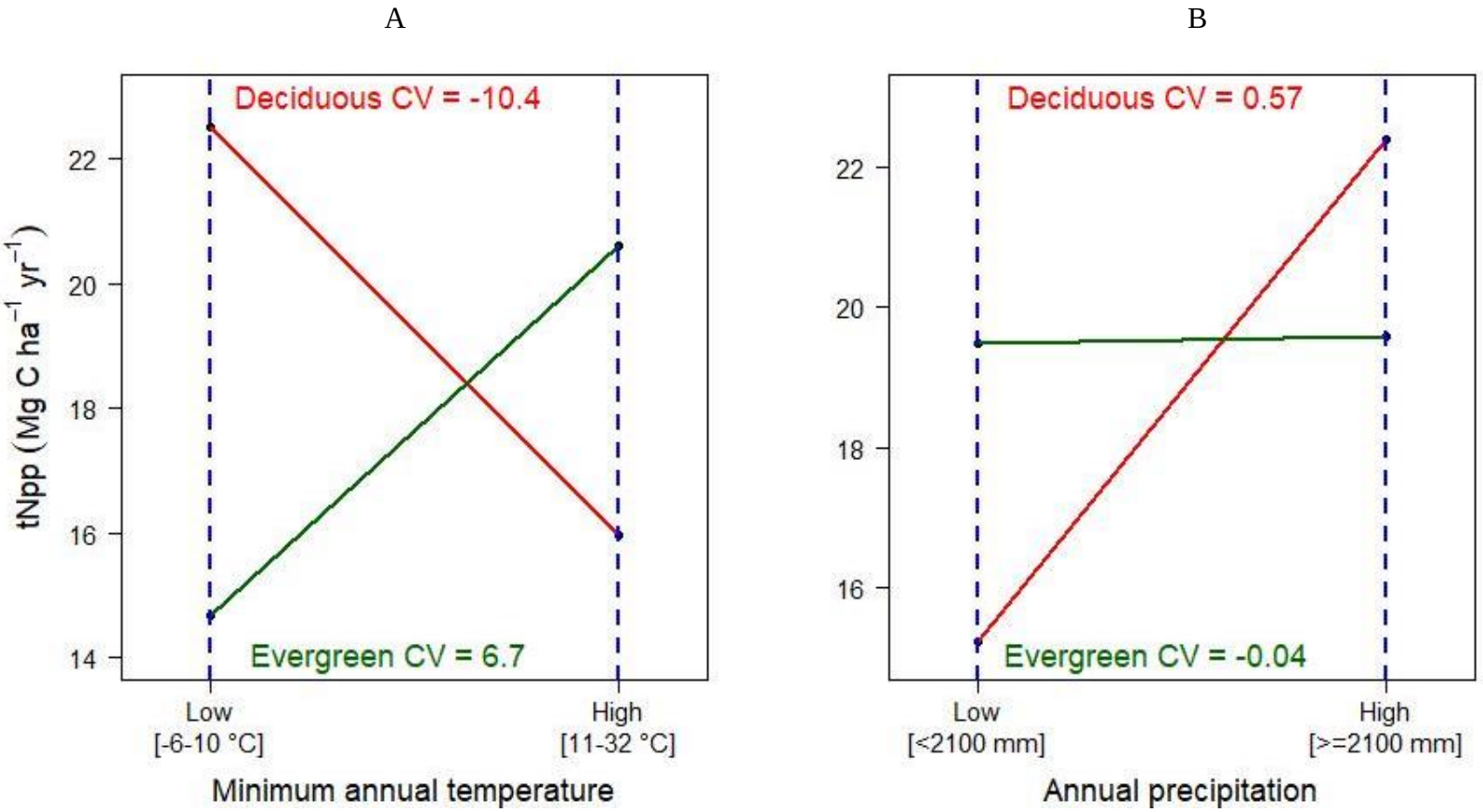


Figure 4-22: Reaction norm plots for tropical forests. Phenology shows an interaction and dependence of tNpp on (a) minimum annual temperature and (b) annual precipitation. Deciduous forests (a) decrease 7 Mg ha−1 yr−1 with high minimum annual temperature and (b) increase ~7 Mg ha−1 yr−1 with high precipitation levels, as evergreen forests (a) increase and (b) are flat.

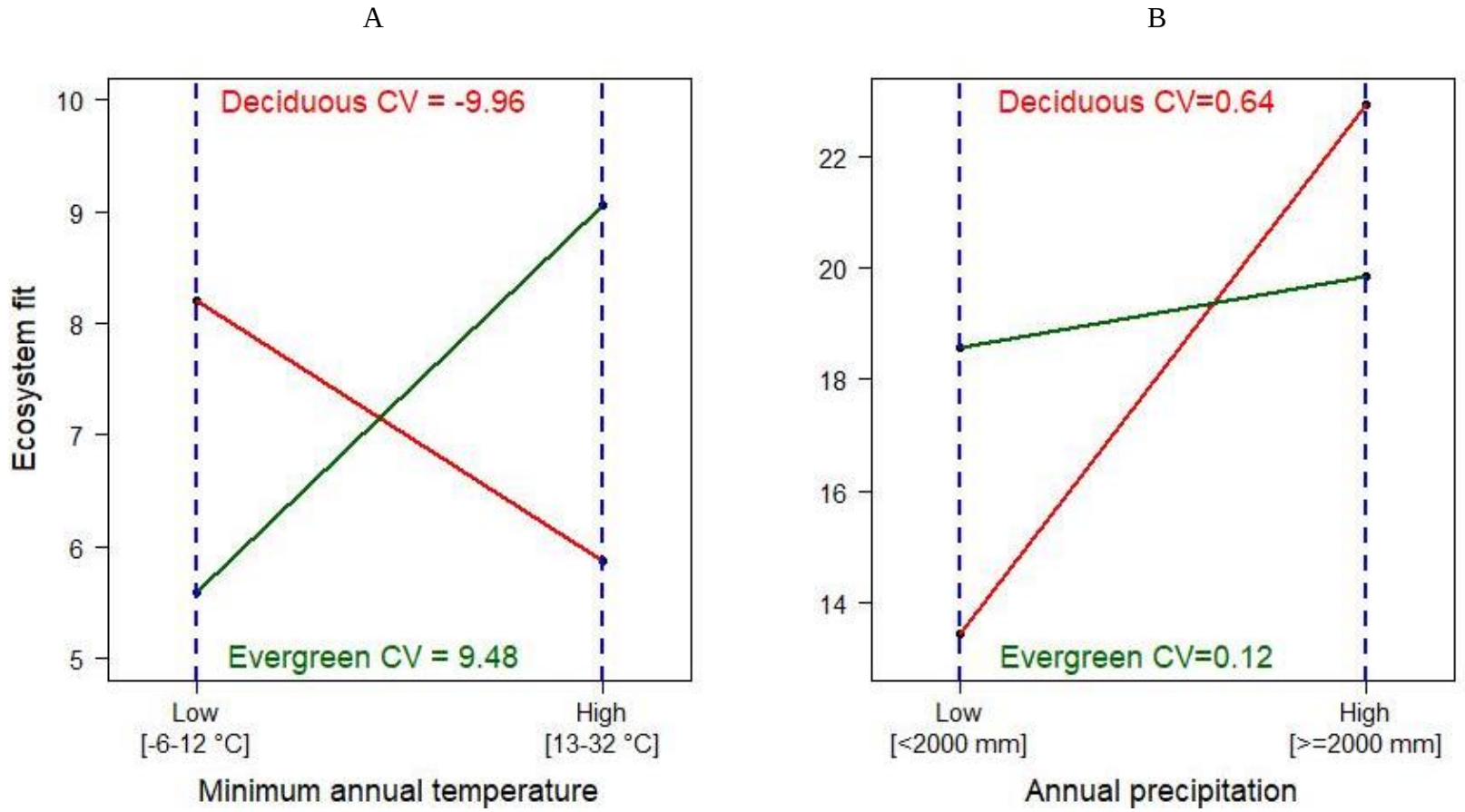


Figure 4-23: Reaction norm plots for tropical forests. Phenology shows an interaction of Ecosystem fit (eFit) to (a) minimum annual temperature and (b) precipitation. Ecosystem fit (A) declines 2.5% for deciduous forests at the high range of minimum annual temperature and (B) increases almost 9% at high levels of precipitation. Evergreens increase 3.5% with higher minimum annual temperature and eFit increase ~ 1% at the high level of precipitation. The influence of phenological traits on eFit in tropical forests is variable and dependent on trait interactions with the environmental variable ranging from 1-10%.

Global tNpp analyses identify climatic factors as explaining a significant portion of global carbon sequestration rates. For example, Luyssaert et al. (2007) showed that climatic factors explained 71% of the variability in GPP using a meta-analysis of 513 different global data sets of forest carbon budgets from the boreal, to temperate and tropical regions. Since temperature is also the threshold at which the productive capacity of forests begins to decrease as respiration rates increases and higher rates of water uptake are needed to keep leaves cool, these correlations are significant at the biome level of analyses. Frequently elevation is identified as an important variable in biome scale assessments of tNpp which identifies changes in vegetation community growth along altitudinal gradients (e.g., Propastin, 2011). These elevation changes reflect strong climatic variances with elevation, but they also demonstrate changes in productivity related to soil fertility as well as climatic factors. Development of models has been challenged by the need for field parameterization, calibration and validation at the site scale which is challenging since data does not exist in many parts of the world as input to these models (Zheng et al., 2003). These temperature thresholds identified at the global scales are useful to categorize sites and their vulnerability to climate change, but they do not provide mechanistic explanations for why a forest is unable to adapt to environmental change.

The various combinations of factors controlling tNpp at each site means that modeling carbon sequestration rates of forests using GPP may under- or over-estimate tNpp at the site scale. The models are being developed that can include multiple combinations of factors but few edaphic controls on GPP and tNpp (e.g., Huston & Wolverton, 2009). The MOD17 algorithm estimates GPP and NPP using Fraction of Photosynthetically Active Radiation (FPAR), Leaf Area Index (LAI), temperature, incoming solar radiation, vapor pressure deficit, land cover classification, biome specific respiration calculations, vegetation types (Running & Zhao, 2015). Model projections of productivity changes with the projected temperature and precipitation changes (Christensen et al.,

2019). The original equations developed a linear relationship between the amount of solar energy absorbed by crops not limited by water or nutrients (Running & Zhao, 2015). It doesn't include how forests adapt to the limitations of the growth environment.

Recently, Battipaglia et al. (2020) wrote how a vulnerability assessment combines how species adapt as well as the measure of the potential impact of a stressor. They further summarized the following research gaps: (1) Better integration of direct observation, field data and modeling, (2) Lack of studies on belowground C allocation in response to changes in temperature, water availability and management practices; and (3) "linking above- and below-ground growth dynamics in the continuum soil-plant-atmosphere." These models tend to assume that ecosystems are stable and do not change functionally and structurally with time. Also, relationships used to predict tNpp focus on the physiological controls on photosynthesis and do not adequately include the soil and microbes that determine how well vegetation are able to compensate the site constraints to growth. A plant physiological approach to estimating tNpp may establish correlations that drive plant production, growth, and maintenance relationships that does not include a multiple of other factors that determine carbon allocation to all these processes.

4.2. Adaptation and Environmental Heterogeneity

Environmental heterogeneity drives adaptation and evolution (DeWitt & Langerhans, 2004). The diversity of forest ecosystems is correlated with sustained but moderate environmental or ecological challenges where the responses exhibit phenotypic adaptations that are temporally dynamic within the climatic region in which they evolved (Dewitt, 2016).

Tree leaf phenology, deciduous vs evergreen is an evolutionary divergent strategy resulting from trade-offs that differ according to the unique combinations of the experienced abiotic and biotic conditions. Information on tree leaf phenology was included in estimates of biome-level

tNpp assessments since they broadly reflect how trees have adapted to their growth environments and are strongly controlled by climatic factors. Deciduous trees drop their leaves in response to drought or cold temperatures and have evolved in response to seasonal predictability as new resources have reliably became available to regrow leaves every year. Deciduousness is successful in many different types of conditions and climates. Generally, evergreen trees have lower carbon-capture capacity per unit leaf area than deciduous trees as they require higher carbon allocation to grow and maintain structural tissues (Smith, 1993). In response to this demand, evergreen trees have evolved multiple compensatory mechanisms. These include the ability to store and mobilize nutrients to areas of active growth, higher total leaf area (Sprugel, 1989), multiple cohorts of annual leaves, leaf shape that allows sunlight to penetrate to lower branches and shed snow, and a conical tree morphology. Evergreen trees typically grow on nutrient-poor soils (Grime, 1983) whereas deciduous forests typically grow on nutrient-rich soils (Reich et al., 1992). This is not the rule and interesting dichotomies are frequently observed. For example, evergreens are favored in wet tropical soils that are continually leached but have high remineralization (decomposition) rates and are also found in boreal climates where nutrients are bound up in the soil and generally unavailable as remineralization rates are slow. Tropical and boreal climates share nutrient impoverished soils, but the evergreen life history strategy works well in both.

Nutrient availability also differs temporally as deciduous forests often experience a seasonal flush of nutrients in tropical and temperature climates whereas evergreen forests experience a gradual release of nutrients throughout the year in the tropics and most of the year in colder climates (Smith, 1993; Kaluthota et al., 2015). Trees also exhibit photosynthetic latitudinal adaptation whereby deciduous trees in higher latitudes have higher photosynthetic capacity as a possible compensatory mechanism for a shorter grow season and/or reduced insolation (Kaluthota et al., 2015). Conversely, cold climate evergreens do not photosynthesize all year but may benefit from a

longer season to gain carbon as they retain their leaves (Waring & Franklin, 1987; DeWitt & Langerhans, 2004). These mechanisms are examples of morphological and physiological adaptation in response to direct environmental forcing. Leaf phenology knowledge is insufficient to model how much carbon is allocated to bNpp or tNpp, nor does it identify the edaphic constraints to growth at the site scale.

Plasticity is how organisms respond to changes in the environment to improve resource-use efficiency (Tchokponhoué et al., 2019). The adaptations expressed by the organisms to the normal range of climatic conditions of their ecosystem has a direct and robust relationship with ecological resilience (Anjos & De Toledo, 2018). Plasticity can also be viewed as a measure of environmental sensitivity when productivity is constrained by some environmental condition.

Observations of tree phenotypic plasticity have been made in every biome and it is one of the most efficient ways that trees can cope with environmental and ecological stressors (Laurans et al., 2012, Gratani, 2014). In the evolutionary context, phenotypic plasticity demonstrates two examples of adaptation, first, plasticity is a heritable adaptive trait that varies in strength of gene expression, and second plasticity increases fitness (not eFit). We understand that plasticity increases a trees ability to adapt, but it is important to separate inferences about the strength of the environmental selective pressure on the trait (e.g., timing of spring bud break by photoperiod) to the plasticity of that trait (e.g., delay of spring bud break due to cold temperatures). For example, tree species conform to an average growth height that has evolved as a series of tradeoffs to balance environmental/ecological limitations and how best to allocate resources to growth, maintenance, and reproduction (non-plastic threshold response). Conversely, young trees generally grow taller in crowded conditions, which is adaptive as it increases a saplings' competitive ability to acquire sunlight (plastic response to competition).

The plasticity of trees to modify their physiology and morphology in response to environmental cues will be critical in the context of rapid climate change. Native forest tree species that have high adaptive plasticity which tends to stabilize productive capacity may have advantages with environmental forcing and emerging threats such as novel pests (Kijowska-Oberc et al., 2020). Forests that are physiologically able to maintain efficient resource-use will have a survival advantage as the system moves away from the optimal climatic conditions.

Calculating a coefficient of the range (CVr) is useful to explore how well adapted forests are to their site at the biome scale since CVr is the ratio of the difference of the growth response to the sum of the maximum and minimum values of the response. For this study, CVr was calculated for tNpp and eFit to evaluate the amount of variation among each response in each climatic zone. The results of this study indicated that temperate forest had the highest CVr values for tNpp and eFit indicating higher variation in both "effect" traits (traits that determine effects of trees on ecosystem functions). A higher value indicates that temperate climate forests are less stable in the range of the response and therefore more capable of adaptation to environmental variations. Tropical and boreal forests had lower CVr values indicating that these forests show less variability in range and may be less capable of strong selective pressure that drives their productivity very high or very low (Table 4-2). A comparison of the NoR for low to high threshold values of temperature showed monotonic decreases in tNpp for all deciduous forests (mean ~ 5 Mg ha^{-1} yr^{-1}) except the boreal climate, and monotonic increases of tNpp in all evergreen forests.

The RF classification model was used to identify variable importance and which variables were the most influential predictors. In this study, this model identified four environmental variables and two soil orders whose importance were deemed to contribute significantly to the classification of boreal, temperate, and tropical forests. This demonstrates how robust the identification of high-level forest types can be predicted with few variables and minimal field-

collected data (Fig. 4-14). Deciduous and evergreen forests were the two most influential environmental gradients to compare the behavior of each effect trait by climate and phenology. Total net primary productivity was strongly dependent on temperature and precipitation in all climatic regions and elevation having influence in the temperate zone. Elevation to 750 m exerted positive dependence on tNpp in the boreal climate but productivity declined steadily with increasing elevation in the temperate zone. We calculated the Coefficient of variance of the means (CV_m) for four ecosystem properties to minimum annual temperature to determine if any of these variables varied within each climatic group (Table 4-8). The asymptotic test statistic for tNp was 6.758, p = 0.079 for tNpp and 7.419, p = 0.059 for eFit indicating no difference in variation in any climate zone. Since this study has shown that variations in the edaphic constraints determine the level of tNpp and eFit value attained, this supports the use of local-scale analyses to assess whether forests are vulnerable to climate change.

Table 4-8: Summary of coefficient of variation of the means (CV_m) representing our plasticity index for four physiological ecosystem properties. Monotomic increases and decreases in functional trait mean indicated by "+" and "-" respectively. A Kruskal-Wallis test was applied to the functional trait means across the two thresholds for minimum annual temperature corresponding to low and high levels. Level of significance; * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$, **** = $p < 0.001$.

		Total net primary productivity (tNpp)				Photosynthetic efficiency (E_c)			
Climate	Leaf phenology	Mean at low min-temp	Plasticity index	SD	*p*	Mean at low min-temp	Plasticity index	SD	*p*
Boreal	Deciduous	11.5	-15.2	3.5	**	0.071	0.5	0.002	
	Evergreen	4.3	11.2	2.5	***	0.070	0.4	0.002	***
Temperate	Deciduous	14.6	-2.7	3.8		0.069	-1.2	0.002	****
	Evergreen	13.4	1.5	5.4		0.071	0.1	0.003	
Tropical	Deciduous	22.5	-10.4	3.3	**	0.059	-1.5	0.005	*
	Evergreen	15.8	6.7	7.6	*	0.060	-2.0	0.004	****
		Allocation below ground (AbNpp)				Ecosystem fit (eFit)			
Boreal	Deciduous	23.9	4.6	7.7		9.9	-21.8	3.68	**
	Evergreen	31.2	-9.2	10.6	**	3.6	6.5	2.22	*
Temperate	Deciduous	31.1	-0.3	21.9		7.6	-10.9	2.23	***
	Evergreen	28.7	1.6	17.4		6.1	0.5	2.55	
Tropical	Deciduous	31.4	0.5	15.3		8.8	-10.0	1.60	**
	Evergreen	50.5	-0.3	12.8		6.1	9.4	2.96	**

4.3. Edaphic Constraints to tNpp and eFit

Environmental conditions form gradients of conditions which ebb and flow across the landscape, but constraints are not mutually exclusive and often occur simultaneously, varying over time and space, and ecological levels of organization. For example, at the micro-scale the relative proportion of sand, silt and clay minerals, and the degree of soil development are determinative of soil nutrient availability and water holding capacity. The structure of the soil exerts strong control over the community composition of microbes that in-turn determine the rate of remineralization of organic matter according to moisture, temperature, and oxygen. Edaphic variables including pH are strongly determinative of nutrient availability, such as occurs in acidic peat or clay soils, or high pH soils developed from old seabeds, calcium carbonates, or other salts which impose site-level limitations to growth. Soil pH affects bacterial diversity (Fierer & Jackson, 2006) and the relative growth-rates and abundance of bacteria and fungi (Rousk et al., 2009). The species composition of the soil community determines decomposition rates (Maron et al., 2011) and bacterial diversity is correlated with the colonization of roots by arbuscular mycorrhizal fungi (Ferreira et al., 2020). Forests rely on close symbiotic associations with mycorrhiza fungi as these organisms are essential to providing water and important limiting minerals such as phosphorus to the roots. In turn, microbial metabolism changes soil characteristics and can act as a buffer to the soil pH.

Tree species control most of the community structure and ecosystem properties of forests (Ellison et al., 2005) and the environmental context in which forests evolved vary from favorable to extreme, stable, seasonal and/or stochastic, and nutrient-rich to nutrient-impoverished (Dewitt, 2016). All forests face similar challenges and have developed a core set of effect traits that characterize how they respond to environmental conditions existing at the local site. Environmental factors are strongly coupled to forest species composition and determined by natural selection over

evolutionary time in conjunction with the stability of site-level conditions (Shugart, 1984). Forest ecosystems must find the perfect balance between multiple competing demands for growth, obtaining nutrients, and coping with water stress. Leaf phenology is a morphological trait that evolved to cope with regional conditions; deciduous forests drop their leaves each year and evergreen forests keep their leaves throughout the year, where each strategy has settled on a different, but equally successful, cost/benefit ratio. In some regions evergreen forests predominate, sometimes deciduous forests do, but most of the time these two phenological strategies coexist. Forest ecosystem properties are phenotypically plastic and can respond to different ecological settings. For example, tree growth may be stunted when grown in habitats other than the one to which they are adapted. However, when trait expression in different habitats has been shaped by natural selection, phenotypic plasticity represents an ecological strategy that is adaptive to environmental heterogeneity and forms a framework for the building of ecological relationships (Agrawal, 2000).

In global tropical forests, Vogt et al. (2016) reported how a multiple of diverse combination of factors explained the same field-based tNpp achieved on a site and the existence of several thresholds of Npp driven by precipitation levels. The thresholds of low, medium and high tNpp showed that within each tNpp level, the low and high tNpp groups had a lower diversity of variable combinations explaining productivity suggesting fewer adaptive mechanisms by the primary producers and their symbiotic partners to their growth environments – in one case too much water and in the other case too little water to produce tissues, to grow and to maintain their tissues. These mechanistic combinations of correlations explaining tNpp at a local site cannot be detected using only an aboveground focus, since plant adaptation frequently occurs as shifts in carbon allocation to the organs or symbionts that are the most limiting to growth (Waring, 1987). One needs to

recognize that aNpp and bNpp is part of adaption and how species adapt to a changing environment – it is the biodiversity strategies found in trees (Gordon et al., 1993).

The variance and heterogeneity of local environmental conditions to which trees adapt, are needed to be able to predict site-level tNpp and Ecosystem Fit data. Further, management decisions occur at the local scales where differences in soil types varies across very small scales and impact the tree species found growing on a soil (Davies et al., 2005; Palmiotto et al., 2004; Paoli & Curran, 2007). Researchers working in the temperate and tropical forests have also reported how a forest ecosystem varies structurally and functionally responding to local variations in resources and site conditions, e.g., edges of forests having more light penetration into a forest resulting in drier conditions (Franklin, 1987; Vogt et al., 2016). A forest is more than the sum of the trees and the climatic conditions. To assess the vulnerability of an ecosystem to climate change and land use changes must occur at the local level where vegetation adapts to site constraints. Juxtaposed at the regional-scale, climate and solar radiation, washes over the geological and topographical features of the landscape, the latter engaging the former as top-down controls that vary with the seasons.

The importance of identifying eFit and site level constraints to tNpp needs to be part of any analysis assessing the vulnerability of forests to climate change events like droughts. The low recovery rate of Amazon forests reported by NASA (2013) following a drought that had occurred five years earlier attests to the slow recovery rate of these forests. It further suggests that a threshold was surpassed within which the forests normally would adapt and recover. This approach of identifying thresholds would refine carbon sequestration measurements since it would not assume a linear relationship to a variable but specifically addresses that trees can adapt in a range and multiple combinations of environmental variables. When environmental change causes multiple and critical thresholds to be surpassed lower tNpp values and less carbon sequestration becomes a risk in forest

ecosystems. The climate change scenario adds a chronic disturbance that continues to impact forests and potentially reducing their normal range of environmental resilience.

Having such information can be used to develop a reference condition that is possible to determine whether it is realistic to increase carbon sinks in any forest. For example, one may not manage to increase forest productivity if the site experiences water shortages at periodic intervals or a forest is located too far from a river to irrigate a forest. Therefore, if a forest has lost resilience due to past land-uses, it is possible to gauge what an upper threshold is and to determine which the potential is for management to increase the ecosystem benefits from that site. These assessments need to be based on total productivity and not just on aboveground Npp. Net aboveground productivity of plantations in the Philippine, the aboveground net primary productivity (aNpp) of plantation forests was reported to vary from 15-30 Mg ha^{-1} yr^{-1} (Kawahara et al., 1981) which begins to reflect what might be thresholds of productivity possible in this region of the tropics. However, without data on tNpp, it is not possible to identify productivity thresholds since aNpp appears to be increasing but the aboveground productivity may be a re-allocation from the bNpp to aboveground components of trees, e.g., not a real increase in tNpp (Waring, 1986).

4.4. Ecosystem Fit and Within Tree Partitioning to aNpp and bNpp

The vulnerability of forests to a loss of productive capacity in response to climate change needs to measure the part of the ecosystem maintaining a site's productive capacity under a diversity of edaphic and climatic factors. The vulnerability of trees to a loss of productive capacity is most limited by soil nutrients and a soil's ability to store water. In the Vogt et al. (2016) study, tropical forests growing on sandy loam and clay soil textures reached tNpp levels in the Low and Medium tNpp grouping whereas the forests reaching Medium and High tNpp levels were found growing in sand-textured soils. To photosynthesize, leaves of trees need nutrients mostly derived from soils to

produce the enzymes to convert CO_2 to complex carbon compounds. This suggests that the current structure of global carbon models is good at estimating how much carbon or biomass can potentially accumulate in a region or globally but are not sensitive to assess how forests adapt to their site-limiting constraints to growth. These models do not include edaphic factors controlling carbon allocation to roots and mycorrhizas since direct measurements of these variables and relationships data are not available to parameterize and validate the models. Direct measures of tNPP also need to be used to calculate the lower and upper threshold of tNpp using the physical and chemical characteristics of soils.

A large database of field-based research can provide insights to the diversity of combinations since field studies will include many of the site-limiting factors to growth due to land-use change, soil type, soil toxicity and disturbance history that will modulate the expression of climatic controls on the carbon cycles (Proctor et al., 1988; Jha & Singh, 1990; Vogt et al., 1995; Laurance et al., 1999; Potter et al., 1998; Langner et al., 2007; Guo & Gifford, 2002; Girardin et al., 2010; Don et al., 2011). Using a multivariate statistical method on data from 96 tropical field sites where total NPP data was available as well as climatic variables, elevation, soil orders and soil texture classes, Vogt et al. (2016) did identify combinations of climatic or soil factors explaining the multiple and diverse tNpp values reported for a given field site. In this study, the first split explaining total productivity was mean annual air temperature , the remaining significant splits were produced by soil factors. Temperature is important but then it is the edaphic factors found at a site that will determine how well trees are able to adapt to climate change and what their role will be in mitigating its impacts. Therefore, field studies will identify a higher number of variables that control tNpp, many of which might not have otherwise been selected in satellite observations and process models obtained at the higher scales of analysis.

Nitrogen levels have an especially significant impact on how carbon allocation occurs between the above- and the belowground parts of trees, e.g., the higher the soil nitrogen levels at a site, the greater the allocation to photosynthetic tissues. Experimental nutrient manipulations have been conducted in forests to understand how managers can increase aboveground biomass to be harvested. Experimental manipulations are important to determine the causal correlations to determine what drivers will change productivity but typically these experiments are site specific and are difficult to transfer to adjacent sites (*see* Wu et al. 2011). The changes in tNpp allocated to the belowground has been recorded to vary from 31-51% of tNpp when trees grew on nutrient poor sites and from 23-30% when trees grew on nutrient rich sites (Werner, 1989). These reflect the variation of carbon allocation to roots depending on the heterogeneity of nutrient resources available for plant growth at a given site. The model developed by Werner (1989) suggests that there is an optimum foliage biomass at which aNpp is maximized that varies with the site nutrient status.

In an experimental manipulation in New Mexico's Cibola National Forest, *Pseudotsuga menziesii* sites were either fertilized, irrigated, wood chips added to double the C:N ratios and wood chips added and irrigated sites (Gower et al., 1992). This experiment was designed to demonstrate the impact of nutrient and water availabilities on carbon allocation to aNpp, bNpp and tNpp in these forests. These experimental manipulations showed how fine root Npp significantly decreased in the four treatment plots compared to the control sites: 32% allocated belowground in the control site; 19% in the fertilized site and in the wood chip/irrigated plots; 24% in the irrigated site and 24% in the wood chip plots). It showed the importance of water and nutrients as limiting factors to growth in these forests. It also showed how aboveground Npp was 33% greater in the fertilized and irrigation which at the end of the study had significantly increased aNpp compared to the control sites. Similarly, two *Pseudotsuga mensiesii* forests in Washington State, one naturally nutrient rich and the other fertilized, show how tNpp is not significantly increased when there was a significant shift

of carbon to aNpp (Grier & Keyes, 1981; Vogt, 1991). In the fertilized site, the percent allocated belowground changed from 41% to 31% when the site was fertilized with 224 kg/ha of fertilizer but there were no significant changes in tNpp. In a naturally high productivity 40-year-old site, 23% of the tNpp was allocated belowground in contrast to 53% in the low productivity site. These studies support the inherent limitation of increasing sites' total productive capacity since other factors continue to limit growth rates and site productivity.

It is important to recognize that measured increases in aNpp may not represent a real increase in tNpp or to know what variables or combinations of variables limit growth at a site. The importance of carbon shifts between the above- and belowground as well as there is a need to model the diversity of adaptations that forests have to the growth limitations and disturbances at a site (Waring, 1986; Vogt et al., 1996). The range of allocation of carbon to fine roots varies depending on the magnitude of the site limiting factors or which combination of factors are impacting growth (Vogt et al., 1991). Research has shown that a plant allocates carbon to those organs which are involved in the acquisition of the resource most scarce for growth thus causing a reduction in stem growth and a higher allocation of carbon to roots when nutrients are deficient or during a drought (Waring, 1987; Vogt et al., 1996).

Collecting field data on fine roots has been very challenging which is why many researchers have resorted to using ratios or estimating this component of ecosystems. In the past, the lack of abundant field-collected data on aNpp and bNpp meant that these have been estimated or modeled using other ecosystem components that are assumed to be linearly related to changes in belowground biomass during any given year (e.g., Clark et al., 2001; Geider et al., 2001; Battipaglia et al., 2020). Global carbon budgets need accurate bNpp estimates which have not been derived from aboveground and belowground ratios (Ledo et al., 2017; Qi et al., 2019) since they are not sensitive to capturing tree adaptations to a heterogeneity of environmental conditions.

Since plants adapt to a diversity of site scale soil physical and chemical characteristics by shifting carbon allocation patterns between the above- and the belowground tree components to mycorrhizal associations, using gross carbon budgets to determine the upper bounds of belowground NPP levels for a site does not capture the multiple adaptive processes that explain these allocation shifts, or which combination of site level factors are early warning indicators of changes in tNpp. Many of the belowground responses to environmental change are early warning and sensitive indicators to system level changes that are not visible or detected from measures of aboveground components of ecosystems (Vogt et al., 1993).

4.5. Plantations and Natural Forests: Potential tNpp Increases by Alleviating Site Limitations to Growth

Plantation forests can be used to determine whether forest management can alleviate the limiting constraints to growth to increase tNpp beyond unmanaged sites. Since the Theoretical Productivity Potential is estimated using variables that are not internal to the site, this is a form of "precision forestry" where plantations are managed to eliminate natural environmental limitations that would reduce growth. This has implications for plantation managers as traits for plasticity can be exploited to improve productivity in various climatic and edaphic conditions. Understanding the site-level adaptation of trees is crucial to predicting the resilience of forest ecosystems. But how do we distinguish between high levels of local adaptation and phenotypic plasticity, to the odds of survival under future climatic conditions?

In this study database from plantation forests, the percent of tNpp allocated to roots varied from 5 to 50% for plantations being managed mainly to increase aboveground biomass. Most of the plantation sites did not have a significantly different percent allocated to the belowground compared to natural forests growing in the same location. Plantations in India had from 12 to 23% of total

Npp allocated belowground (n = 24), those in China varied from 26 to 50% (n = 6), whereas Brazil plantations had from 32 to 56% of total Npp allocated belowground (n = 3). Plantations in North Carolina (n = 1) and Florida (n = 1) had 28-29% of productivity allocated belowground although the lowest level was in New Zealand (5%; n = 2) in naturally fertile sites with extensive root grafting.

The highest aNpp levels have been recorded in plantation forests that are managed to increase tree growth rates. These studies, however, do not inform on the potential maximum tNpp that can be reached in plantations. A significant increase in aNpp have been reported for many plantation forests. Net aboveground productivity of plantations in the Philippine tropics varied from 15-30 Mg ha^{-1} yr^{-1} (Kawahara et al., 1981) which begins to reflect what might be thresholds of productivity possible in this region of the tropics. In Brazil, Stape et al. (2010) studied the productivity potential of Eucalyptus plantations grown at different fertilization and irrigation conditions even though the data only measured aboveground productivity. These data are still useful to see how much aNpp can increase at any site and what factors can be alleviated to move forest stands closer to its optimal productivity potential. For example, Eucalyptus plantations on drier sites increased aNpp from 25 Mg ha^{-1} yr^{-1} of stem wood production before irrigation to an optimal 42 Mg ha^{-1} yr^{-1} (Stape et al., 2010). According to the summary by Stape et al. (2010), managing water supplies to Eucalyptus has resulted in stem growth increases that were 35% higher than in unmanaged plantation forests.

The general trend is that as increases in aboveground stem growth increases less is partitioning of biomass occur into roots. Previous research summarized in Vogt et al. (1997) included data on plantation and natural forests in the tropics and how much carbon was allocated to fine root biomass. In the tropics, there was a general pattern of less total live root biomass (4-11%; n = 13) in plantation forests compared to natural forests growing in the same area and edaphic environments (6-33%; n = 11). In a meta-analysis of the impact of drought stress on biomass

allocation, Eziz et al. (2017) reported that drought stress significantly increased the carbon allocation to roots but decreased carbon partitioning to aboveground stem, leaf, and reproductive structures. This means that any increases in total productivity are lost as trees adapt to droughts. The shifts in carbon allocation to aboveground tree components occurred when a limiting resource was eliminated through management.

The alleviation of some site internal factors by irrigation or the application of fertilizers shows how plantation forest growth rates are not predicted by local scale internal site factors. In this study, the various combinations of factors that determined natural forest growth did not predict plantation tNpp or to aNpp. Like natural forests, plantation forests had a significantly lower Npp in roots when the sites were amended with either fertilizers or irrigated to compensate for the site's water limitations (Litton et al., 2007). In this study's database, when plantations were not fertilized or irrigated to alleviate limiting resources, tNpp of plantation forests did not significantly vary from what was recorded in natural forests. These stands showed that there was a higher potential tNpp but there was also less of tNpp partitioned to the belowground components of a forest. Since there is less need to allocate carbon to roots to acquire water and nutrients, carbon allocation reflects this.

Thus, plantation forests growth rates are a useful lens to gauge what are potential optimal tNpp values for a local area despite only reporting aboveground productivity data. Since managers alleviate variables that can limit annual growth, it can identify what is the potential to increase tNpp for a site and therefore identify an optimal productivity threshold. Some of the local environmental variables will still limit the optimal tNpp that can be managed for since not all variables can be controlled or alleviated, e.g., temperature or precipitation. The results of our database analyses did show that climatic factors did appear to limit the growth rates of plantation forests more significantly. For example, in our medium Npp group where maximum air temperatures were higher than 26.7 °C, total Npp values were at the intermediate level for sites growing at elevations of

280 – 519 m. To reach the high total Npp levels in plantation sites, this database suggests that trees need to be planted in areas where the maximum air temperatures are lower than 26.7 °C. This means that even plantation forests that are being managed have thresholds beyond which the total growth cannot be increased. Plantation forests can also suggest the reference condition that a manager can aim for while rehabilitating degraded lands especially for obtaining watershed protection and ecosystem services (Parrotta et al., 1997).

Plantation forests are decoupled from their environment when they are fertilized and irrigated to adjust for site deficiencies in resources (Cossalter & Pye-Smith, 2003). Therefore, variables that explain changes in biomass and production in natural forests should not limit the growth rates of plantation forests if management has correctly determined what limits growth. However, plantation forests managed to increase stem growth with less biomass partitioning to the belowground, will be more vulnerable to climate change impacts unless managers are able to continue to irrigate forests. Poorter et al. (2012) reported that plants partition more biomass to roots when water levels limit growth such as in a drought. Further, if fertilizers are added to increase aNpp in plantations, trees will lose their mycorrhizal associations (Lilleskov, 2008) and the occurrence of droughts means that trees will lose some of the other benefits of these symbionts and fine roots. This would make plantations more vulnerable to climate change if management is unable to continue to alleviate the site limitations to growth.

Nutrient manipulation experiments that measured aNpp and bNpp support the fact that eFit represents the constraints that limits the potential increases in tNpp achievable at any given site. The general trend is that the lower the nutrient status of a forest, the higher the allocation to fine roots and mycorrhiza, and that tNpp is a result of allocation shifts between the belowground and aboveground portions of a forest. In *Pinus resinosa* plantation forests in Wisconsin, the addition of soda lime suggested that roots and mycorrhizae contributed 21 to 30% of total soil respiration in

unfertilized red pine plantations in contrast to 4 to 12% in fertilized forests – a significant decrease in bNpp due to these additions (Haynes & Gower, 1995).

Having such information can be used to develop a reference condition that is possible to determine whether it is realistic to increase carbon sinks in forests. One may not pursue this possibility because of water shortages, or a forest being located too far from a river, but it does suggest a threshold for any forest if it is not decoupled from the local environment. Therefore, if a forest is less able to adapt to changes resulting from past land-uses, it is possible to gauge what an upper threshold is and to determine which sites might have the potential to be targeted for management to acquire ecosystem service benefits from forests.

Plantation forests are not resilient compared to natural forests and plantations are therefore less able to adapt to their environment. Use of aboveground data from plantations must consider the fact that plantations appear to sequester less carbon in live belowground parts of trees (Vogt et al., 1996). Plantation forests are not adapting to their site since their limits to growth are intensively managed. If plantations are better at growing aboveground parts but have a low belowground biomass, their resilience in the face of disturbances will become an issue in the future if management practices cannot be continued.

4.6. Paleo- and Neo-tropical Forest tNpp and eFit

The general patterns of how carbon accumulates in forests are general in the sense that it is easy to identify the pools and fluxes of carbon which models are based on. However, spatial and temporal dynamics and variations in the magnitude of these pools and fluxes are site specific. This was reflected in our database by exploring for patterns in the tropical region and did show how the achievable tNpp will vary across different regions of the tropics where the Asian tropics have higher

tNpp in natural forests compared to the Neotropics but the Americas have higher tNpp in managed forests (Tables 4-3, & 4-4, Fig. 4-5).

A recent comprehensive review by Fiji et al. (2018) described and explained the distribution patterns that our analyses produced. Their study described how variations in vegetation, soil animals and microorganisms resulted in different tree species and different patterns of biomass in different regions of the tropics all growing on highly weathered soils. They described how forests growing in the Southeast Asia region are dominated by dipterocarps growing on soils that are acidic Ultisols but with young parent material in contrast to the Neotropics and African tropics where there are many leguminous trees that grow on Oxisols formed from old parent materials. They further wrote how ectomycorrhizal fungi allow dipterocarps to acquire limiting supplies of phosphorus for the mast fruiting to occur on soils with limited P supplies. In contrast, Oxisols have limited P availability compared to N supplied by leguminous trees showing the importance of soil weathering impacting the distribution and growth of vegetation. These differences are more difficult to capture in a generalized process model and at the local scale.

This study's analyses showed that both the Paleo- and Neo-Tropical forests had the same eFit but the Paleo-Tropical forests had significantly higher tNpp. This suggests that the Paleo-Tropical forests had a greater range of adaptations to their site despite both having the same theoretical maximum productive capacity. If these sites were compared using models, these differences would probably not have been detected or identified.

This research showed using field collected tNPP data that the Paleotropics have a significantly higher tNPP compared to the Neotropics. Using only aboveground biomass and Npp data, Taylor et al. (2019) produced similar results where stem growth was higher in the Paleotropics and aboveground biomass was 29% higher despite having similar climates. This supports the role of the edaphic and genetic factors in determining growth rates in the paleotropical forests. Their

comment, on land models treating paleo- and neo-tropical forests as being similar despite having significantly higher growth rates, supports the use of the Ecosystem Fit model to assess the vulnerability of forests to climate change or other disturbances.

4.7. Forest Adaptation to Environmental Heterogeneity and their Vulnerability to Climate Change

In our database, leaf phenology was responsive to the environmental gradients of temperature and precipitation. For example, tNpp improved for deciduous and evergreen forests as minimum annual temperature and annual precipitation increased. Evergreen forests generally had lower tNpp across all ranges of precipitation but evergreen forest tNpp outpaced deciduous forests at the high-level temperature thresholds. This crossing of the lines indicates an interaction between the environment and the phenological trait. These aggregated forest plots showed that evergreen forests had larger tNpp plasticity and were better able to respond to changing environmental conditions than deciduous forests. The effect was much more subtle with precipitation.

Photosynthetic efficiency was similarly responsive to the environmental gradients of temperature and precipitation in a purely linear environmental context. Efficiency declined for deciduous and evergreen forests with increasing temperature and precipitation with a steeper increasing slope for temperature. Deciduous forests had slightly lower efficiency across all ranges of the variables. This illustrated that the physiological trait of efficiency was plastic in relation to the environment but not to phenological structure. Therefore, deciduous and evergreen trees should show similar response across temperature and precipitation gradients.

Interestingly, AbNpp interacted with minimum annual temperature and leaf phenology but not decidedly for precipitation. The physiological processes that drive allocation of resources are known to be highly plastic and a key evolutionary mechanism of adaptation. Belowground

allocation increased with both variables for deciduous and evergreen forests but the slope for plasticity for AbNpp reversed between temperature and precipitation. There was a steeper slope for evergreen forests with temperature whereas deciduous forests demonstrated hard saturation, and conversely, deciduous forests had a steeper slope with precipitation, but the NoR of evergreens was saturated. The intersection of the NoR with temperature represents a strong interaction between phenology and the processes of allocating resources to roots. This comprises a critically important physiological effect trait that is strongly responsive to site-level conditions and has high adaptive value. This interaction tells us that evergreen trees are more resilient to higher minimal annual temperatures.

Across mean annual grow temperature, we can see that the physiological plasticity of productivity among tropical forests was greater than temperate or boreal forests. We can compare the three climatic regions although they do not occur across the gradient as they have different temperature optimums. Plasticity for the trait of productivity may allow forests to grow in more heterogeneous habitats. Physiological plasticity is linked to an enhanced ability to rapidly colonize open areas such as gaps from old-growth tree fall, fire, or other disturbances the open the canopy. Physiological plasticity is more readily demonstrated in many trees that genetic constrained morphological and anatomical plasticity. Trees growing in stress conditions tend to have a conservative leaf morphology to reduce unnecessary production of structures that are resource intensive and unable to be sustained (Cunningham et al., 1999). Morphological plasticity is enhanced in understory shrubs and small trees as resource acquisition is paramount (Letts et al., 2012).

Forests exhibit an array of environmentally induced responses that have different reaction times depending on whether they are physiological, morphological, or behavioral. These phenotypic variations may include changes in timing for new growth and reproduction, changes to how

resources are allocated to roots or other tissues, or changes to the size and shape of leaves. In general physiological adaptations operate on shorter time scales than morphological (Pigliucci et al., 2006). Strong variance of phenotypic expressions are adaptations to short-term environmental fluxes, and it seems likely that any phenotypic plasticity that positively correlates forest productivity with climate predictions will be critical for adaptation to novel conditions within the time frames.

Seasonality exerts strong divergent selective pressures on tree natural history where the alignment between the phenotype and the environment are crucial for successful flowering, seed dispersal, or setting of fruit. In response forests show widespread adaptive effect traits to seasonal variation as they track the predictable periodicity of contrasting environmental conditions. If seasonally initiated physiological responses of a forest are too rigid and unable to respond to climatic conditions (i.e., outside the normal SOS) there may be temporal mismatches significant enough to reduce fitness. What remains unclear is to what extent and under what conditions is there enough flexibility in seasonal adaptations for long-term survival or are they so inflexible that they limit adaptation (Oostra et al., 2018).

Many factors influence how carbon is produced, used in the construction and maintenance of tissues that are not linearly related to one another. There is value to use the field site data to identify which variables are controlling tNpp or partitioning between aNpp and bNpp. Some of these variables are controlled by symbionts such as toxic chemicals in the soil, low soil delivery capacity of nutrients so fungi mineralize soil available limiting nutrients by releasing oxalic acid, defending trees against pathogens emerging from the soil (Cromack et al., 1979). Some attributes of plants may be identified by comparing evergreen versus deciduous trees and their strategies for acquiring resources to grow (Franklin & Waring, 1979). The symbionts found at any site are diverse and change with plant successional stages and age (Frankland, 1992), phenotypic plasticity of root growth form depending on site characteristics, disturbances, like wind, causes increased allocation of

carbon to structural roots. Northrup et al. (1995) reported on how mycorrhizas control nitrogen release from pine litter.

5. Conclusion

Models should be used to explore and identify which regional landscapes might be sensitive to climate change or other disturbances but are not designed to derive local management practices. The ecosystem fit allows a manager to get a better perspective of factors that will constrain management options. Our goal should not be to use models to measure ecological processes over larger scales since this would generalize the adaptive mechanisms and lose knowledge of what factors may decrease management options. This would suggest that field-based data and models should be used as the first level of filters to form knowledge of the impact of a local forest to climate change. Ultimately at the local level, managers who know the landscape and have a longer-term history of the land should be the ones who determine how to use forests to mitigate the impacts of climate change. Assessments done at the larger scales are unable to factor in local factors that may limit management options or should make decisions on what should happen at local scales. This is similar to wildlife management that was more successful when local communities impacted by the decisions were co-managers of land-use decisions (e.g., Conniff, 2013).

As stated by Landsberg et al. (2020) and many others, models may need to be more sensitive to the multiple factors that are site specific and internal to a site. This would make a model reflect the variable combination of growth limiting constraints of each site to explain whether a forest is resilient to climate change (maintain or increase its tNpp) or whether climate change impacts can be potentially mitigated in forests. Also, the impact of tree species on tNpp can be seen in the higher productivities recorded for Asian tropical forests compared to the neo-tropical forests in the

Americas in our research. These differences show that it is not just the internal factors that determine the realistic tNpp but differences in tree species dominance at the local site.

Today, the perceived lack of field data from the sites being modeled has focused researchers on the use of global vegetation models to assess the carbon accumulation potentials of forests globally. Models were built using field-collected data from a few sites that represent generalized relationships, but which may not be relevant at the local scale where management needs to know how a specific forest will be impacted by climate change. It is the local scale where trees adapt to a diversity of local scale environmental conditions. This is the scale at which a diversity of symbiotic partners that allow trees to adapt to drought, acquire limiting nutrients needed for photosynthesis and to produce the chemicals to protect their tissues from insect grazers, to avoid taking up toxic chemicals from the soil. It is the local scale where the mechanistic relationships between edaphology, microclimates and symbionts occur that determine the upper and lower thresholds of growth for a tree species and whether it will be vulnerable to climate change. Mycorrhizal associations are essential for forests to adapt to the heterogeneity of site conditions by connecting the belowground structures so that resource acquisition from the soil shared by multiple trees (Trappe & Luoma, 1992; Smith & Read, 2008). There is a reason why more than 80% of vegetation have mycorrhizas on their root systems. Mycorrhizal associations are an important aspect of a safe operating space since they determine the 'ecosystem fit' of the existing ecosystem.

Instead of addressing climate change impacts on forests at the global scale, research needs to address local forest health and whether communities can continue to depend on their forests for their survival or to continue to collect resources for global markets. This need is driven by the significant proportion of the global population that is dependent on forests for their survival and are directly impacted when forest productive capacity is reduced by climate change. Up to 1.7 billion people (200 million Indigenous people) are dependent on forest ecosystems for their survival and

925 million of these people are food insecure (FAO, 2011; Chao, 2012). Whether the diversity of global forests continue to provide sufficient ecosystem services for people dependent upon forests for their survival will be determined by their 'fit' (Gordon et al., 1992) to their growth environment and whether forests continue to adapt to dynamic climates under the continuing constraints of the local edaphic conditions. The indigenous people understand the impacts of the local edaphic conditions on forest growth (Marchand et al., 2020) but their frame of reference is no longer valid since these lands are highly altered today by new land-use practices and climate change.

Managing ecosystems within its acceptable "safe operating space" (Scheffer et al., 2015), or to maintain or to increase the sites productive capacity, will require information on what factors limit tree growth and when do productivity levels decrease sufficiently to make a forest vulnerable to climate change and unable to maintain their productive potential, e.g., shift in vegetative community dominance.

Ecological classifications are typically grouped by similarities along structural, taxonomic, or physiological axes. Ecological fitness is the capacity to optimize productivity in the face of climatic disruption within a defined environmental context. Modern ecological theory proposes three primary types of fitness; the ability to outperform other species (competition), the propensity to develop helpful relationships (commensalism, mutualism), and the "capacity to construct" or to increase the amount of free energy that is circulating within an ecosystem (Peacock, 2011). An increase in free energy is favored by the Second Law of Dynamics in biological systems, as the capacity to increase entropy naturally follows, therefore, each of these types of fitness are favored by natural selection. The capacity of organisms to cooperate becomes increasingly more important as resources become scarce and those resources are more effectively partitioned. For example, in tropical forests soil nutrients are limited but water and sunlight are generally plentiful, this has fostered the evolution of complex ecosystems that optimize subsidies most efficiently and have

evolved complex by-product food webs among soil microbes, mycorrhizae and trees. One of the most important symbiotic relationships are between tree roots and soil microbial communities. In contrast, boreal forests nutrients are generally available but the climatic constraints that limit the grow season and light availability tend to dominate according to Liebig's law of the minimum.

Acknowledgements

This work could not have been possible without the massive effort of gathering the data by Professors Kristiina Vogt and Daniel Vogt. My sincerest thanks for the dataset and insights into "what it all means." I benefitted immensely from our weekly meetings and brain-storming sessions. They taught me to not be afraid to "think outside the tree."

References

Agrawal, A. A., Karban, R., & Colfer, R. G. (2000). How leaf domatia and induced plant resistance affect herbivores, natural enemies and plant performance. *Oikos, 89*: 70–80.

Anjos, L. J. S., & De Toledo, P. M. (2018). Measuring resilience and assessing vulnerability of terrestrial ecosystems to climate change in South America. *PLoS ONE, 13*(3), 1–15. https://doi.org/10.1371/journal.pone.0194654

Battipaglia, G., Rigling, A., De Micco, V. (2020). Editorial: Multiscale approach to assess forest vulnerability. *Front. Plant Sci.*, https://doi.org/10.3389/fpls.2020.00744

Bernacchi, C. J., Pimentel, C., & Long, S. P. (2003). In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant, Cell and Environment, 26*(9), 1419–1430. https://doi.org/10.1046/j.0016-8025.2003.01050.x

Breiman, L. (2001). Random forests. *Machine Learning, 45*(1), 5–32. https://doi.org/10.1023/A:1010933404324

Boyer, J.S. (1982). Plant productivity and environment. *Science, 218:* 443-448.

Bryant, D., Nielsen, D., & Tangley, L. (1997). The last frontier forests: Ecosystems & Economies on the edge. *World Resources Institute. Forest Frontiers Initiative*. Washington DC. Report Number: 149622.

Carpenter, S.R., Cole, J.J., Pace, M.L., Batt, R., Brock, W. A., Cline, T., Coloso, J., Hodgson, J. R., Kitchell, J. F., Seekell, D. A., Smith, L., & Weidel, B. (2011). Early Warnings of Regime Shifts: A Whole-Ecosystem Experiment. *Science 332,* 1079-1082. https://doi.org/10.1126/science.1203672.

Chao, S. (2012). *Forest Peoples: Numbers across the world. Forest Peoples Programme*.1-24. https://www.forestpeoples.org/sites/fpp/files/publication/2012/05/forest-peoples-numbers-across-world- final_0.pdf

Clark, D.A., Brown, S., Kicklighter, D.W., Chambers, J.Q., Thomlinson, J.R., Ni, J., & Holland, E.A. (2001). Net primary production in tropical forests: An evaluation and synthesis of existing field data. *Ecological Applications, 11*(2): 371-384.

Collalti, A., Prentice, E.C. (2019). Is NPP proportional to GPP? Waring's hypothesis 20 years on. *Tree Physiol, 39*, 1473-148.3

Conniff, R. (2013). *People or Parks: The Human Factor in Protecting Wildlife*. Yale Environmental Forum Report. http://e360.yale.edu/feature/people_or_parks_the_human_factor_in_protecting_wildlife/2707/

Cromack, K., Sollins, P., Graustein, W,C., Speidel, K., Todd, A.W., Spycher, G., LI, C.Y., & Todd, R.L. (1979). Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus Hysterangium crassum. *Soil Biology Biochemistry, 11*, 463-68.

Cossalter & Pye-Smith, (2003). Fast-Wood Forestry Myths and Realities. *Forest Perspectives No.1*. Bogor, Indonesia, CIFOR. 54p. ISBN: 979-3361-09-3.

Cunningham, S. A., Summerhayes, D., & Westoby, M. (1999.) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs, 69*(4), pp. 569–588, 1999. https://doi.org/10.1890/0012-9615(1999)069[0569:EDILSA]2.0.CO;2

Cutler, D.R., Edwards, T.C., Jr., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J., & Lawler, J.J. (2007). Random Forests for Classification in Ecology. *Ecology, 88*(11), 2783– 2792.

Davies, K.F., Chesson, P., Harrison, S., Inouye, B.D., Melbourne, B.A., & Rice, K.J. (2005). Spatial heterogeneity explains the scale dependence of the native-exotic diversity relationship. *Ecology, 86*(6), 1602–1610.

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., & Rathgeber, C. B. K. (2016). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. *Annals of Forest Science, 73*, 5-25. https://doi.org/10.1007/s13595-015-04776

Delucia, E. H., Gomez-Casanovas, N., Greenberg, J. A., Hudiburg, T. W., Kantola, I. B., Long, S. P., Miller, A. D., Ort, D. R., & Parton, W. J. (2014). The theoretical limit to plant productivity. *Environmental Science and Technology, 48*(16), 9471–9477. https://doi.org/10.1021/es502348e

Dempster, E. R. (1955). Maintenance of genetic heterogeneity. *Cold Spring Harbor Symposia on Quantitative Biology 20*: 25-32.

DeWitt, T. J., Sih, A. & Sloan Wilson, D. 1998. Costs and limits of phenotypic plasticity. *TREE, 13*: 77- 81.

Dewitt, T. J. (2016). Expanding the phenotypic plasticity paradigm to broader views of trait space and ecological function. *Current Zoology, 62*(5), 463–473. https://doi.org/10.1093/cz/zow085

DeWitt, T. J., & Langerhans, R. B. (2004). *Integrated solutions to environmental heterogeneity: theory of multimoment reaction norms.* In Phenotypic plasticity : functional and conceptual approaches.

Do, T.V., Sato, T., Saito, S., & Kozan. O. (2015). Fine-root production and litterfall: main contributions to net primary production in an old-growth evergreen broad- leaved forest in southwestern Japan. *Ecological Research, 30* (5) 921-930. https://doi.org/10.1007/s11284-015-1295-x

Don, A., Schumacher, J., & Freibauer, A. (2011). Impact of tropical land-use change on soil organic carbon stocks—a meta-analysis. *Glob. Change Biol. 17*:1658–1670. https://doi.org/10.1111/j.1365-2486.2010.02336.x.

Duarte, L.M.L., Rivas, E.B., Alexandre, M.A.V., ßvila, A.Cde., Nagata, T., & Chagas, C.M. (1995). Chrysanthemum stem necrosis caused by a possible novel tospovirus. *Journal of Phytopathology, 143*(9):569-571.

Ellison, A.M., Bank, M.S., Clinton, B.D., Colburn, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloeppel, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N.L., Sobczak, W.V., Stinson, K.A., Stone, J.K., Swan, C.M., Thompson, J., Von Holle, B., & Webster, J.R. (2005). Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front Ecol Environ, 3*(9): 479–486.

Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z., & Fang, J. (2017). Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution, 7*(24), 11002–11010. https://doi.org/10.1002/ece3.3630

Fagan, B. (2008). The Great Warming. Bloomsbury Pres.

FAO. 2011. Forests for improved nutrition and food security. http://www.fao.org/forestry/27976-02c09ef000fa99932eefa37c22f76a055.pdf, accessed 8_6_20.

Ferreira, D. A., Freitas, T., Pylro, V. S., Salles, J. F., Andreote, F. D., & Dini-andreote, F. (2020). Soil Microbial Diversity Affects the Plant-Root Colonization by Arbuscular Mycorrhizal Fungi. *Microbial Ecology*.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology, 37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Field, C.B., & Ehleringer, J.R. (1993). *Questions of scale, p. 1-4*. In: Ehleringer, J.R., and C.B. Field (eds.), Scaling Physiological Processes: Leaf to Globe. Academic Press, San Diego.

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *PNAS, 103*(3).

Firmat, C., Delzon, S., Louvet, J., Parmentier, J., & Kremer, A. (2017). Evolutionary dynamics of the leaf phenological cycle in an oak metapopulation along an elevation gradient. *Journal of Evolutionary Biology, 30*(0), 2116–2131. https://doi.org/10.1111/jeb.13185

Fischer, G., Tubiello, F. N., van Velthuizen, H. & Wiberg, D.A. (2007). Climate change impacts on irrigation water requirements: Effects of mitigation, 1990-2080. *Technol. Forecast. Soc. Change*, *74,* 1083-1107, doi:10.1016/j.techfore.2006.05.021.

Frankland, J.C. (1992). Mechanisms in fungal succession. Pp. 383-401. The Fungal Community. Its Organization and Role in the Ecosystems. Eds. GC Carroll, DT Wicklow. Marcel Dekker, Inc. New York

Friedman, B. J. H. (2001). Greedy Funtion Approximation: A Gradient Boosting Maching. The *Annals of Statistics, 29*(5), 1189–1232.

Garnier, E., Cortez, J., Billès, G., Navas, M.-L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., & Toussaintric, J.P. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology, 85*(9), 2630–2637.

Geider, R.J., DeLucia, E.H., Falkowski, P.G., Finzi, A.D., Grime, J.P., Grace, J., Kana, T.M., Roche, J.L., Long, S.P., Osborne, B.A., Platt, T., Prentice, I.C., Raven, J.A., Schlesinger, W.H., Smetacek, V., Stuart, V., Sathyendranath, S., Thomas, R.B., Volemann, T.C., Williams, P., & Woodward, F.I. (2001). Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global change Biology, 7*: 849- 882.

Geiger, D.R., & Servaites, J.C. (1991). Carbon allocation and response to stress. Pp. 103-127. In: *Response of Plants to Multiple Stresses*. Academic Press, Inc. San Diego, New York, Boston (HA Mooney, WE Winner, EJ Pell, E Chu, eds.)

Genuer, R., Poggi, J.M., & Tuleau-Malot, C. (2019). VSURF: Variable Selection Using Random Forests.

Getty, T. (1996). The Maintenance of Phenotypic Plasticity as a Signal Detection Problem. *The American Naturalist, 148*(2), 378-385. Retrieved June 18, 2021, from http://www.jstor.org/stable/2463460

Girardin, C., Malhi, Y., Feeley, K., Rapp, J., Silman, M., Meir, P., Huasco, W.H., Salinas, N., Mamani, M., Silva-Espejo, J.E., García Cabrera, K., Farfan Rios, W., Metcalfe, D.B., Doughty, C.E., & Aragão, L.E.O.C. (2014). Seasonality of above-ground net primary productivity along an Andean altitudinal transect in Peru. *Journal of Tropical Ecology, 30*(6), 503-519. https://doi.org/10.1017/S0266467414000443.

Gmur, S.J., Vogt, D.J., Vogt, K.A., & Suntana, A.S. (2014). Effects of different sampling scales and selection criteria on modelling net primary productivity of Indonesian tropical forests. *Environmental Conservation 41*(2): 187-197.

Gonçalves, F., Treuhaft, R., Law, B., Almeida, A., Walker, W., Baccini, A., Dos Santos, J.R., & Graça, P. (2017). Estimating aboveground biomass in tropical forests: Field methods and error analysis for the calibration of remote sensing observations. *Remote Sensing 9*(1), 47; https://doi.org/10.3390/rs9010047

Gordon, J. C. & Wheeler, C. T. (1983). Biological nitrogen fixation in forest ecosystems: foundations and applications. (Eds) Martinus Nijhoff / Dr W. Junk Publishers. DOI: https://doi.org/10.1007/978-94-009-6878-3

Gordon, J.C., Bormann, B.T., & Kiester, A.R. (1992). The physiology and genetics of ecosystems: A new target or "Forestry contemplates an entangled bank". *Proceedings of the 12th North American Forest Biology Workshop*. Sault Ste. Marie, Ontario, Canada. Aug 17-20, 1992. Ontario Ministry of Natural Resources, Ontario Forest Research Institute and Forestry Canada, Ontario Region, pp. 1-14.

Gordon, J.C. (1993). *Ecosystem Management: an idosyncratic overview*. In Sample, A. and Olsen, J. eds. Defining Sustainable Forestry. Island Press, Washington, D.C.

Gower, S. T., & Son, Y. (1992). Differences in Soil and Leaf Litterfall Nitrogen Dynamics for Five Forest Plantations. *Soil Science Society of America Journal, 56*(6), https://doi.org/10.2136/sssaj1992.03615995005600060051x

Gower, S.T., Kucharik, C.J., & Norman, J.M. (1999). Direct and indirect estimation of leaf area index, fAPAR, and Net Primary Production of terrestrial ecosystems. *Remote Sens. Environ. 70:* 29-51.

Gosz, J.R. (1992). Gradient analysis of ecological change in time and space: implications for forest management. *Ecol. Appl. 2*: 248-261.

Gratani, L. (2014). Plant Phenotypic Plasticity in Response to Environmental Factors. *Advances in Botany*

Grier, C.C., Vogt, K.A., Keyes, M.R., & Edmonds, R.L. (1981). Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the *Washington Cascades. Can. J. For. Res. 11*: 155-167.

Grime, J. P. (1998). Benefits of Plant Diversity to Ecosystems: Immediate, Filter and Founder Effects. *British Ecological Society, 86*(6), 902–910.

Guo, L.B. & Gifford, R.M. (2002). Soil carbon stocks and land use change: a meta analysis. *Global Change Biology, 8*(4) 345-360. https://doi.org/10.1046/j.1354-1013.2002.00486.x.

Hannah, L., Carr, J.L., & Landerani, A. (1995). Human disturbance and natural habitat: a biome-level analysis of a global data set. *Biodiversity and Conservation 4*, 128-155.

Haynes, B.E., & Gower, S.T. (1995). Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology 15*, 317-325.

Ishwaran, H., & Kogalur, U. B. (2021). Fast Unified Random Forests for Survival, Regression, and Classification (RF-SRC) (R package version 2.10.1). https://cran.r-project.org/package=randomForestSRC

Ishwaran H., & Kogalur, U.B. (2007). Random survival forests for R, *Rnews, 7*(2):25-31.

Hothorn, T., Hornik, K., & Zeileis, A. (2006). Unbiased recursive partitioning: A conditional inference framework. *Journal of Computational and Graphical Statistics, 15*(3), 651–674. https://doi.org/10.1198/106186006X133933

Huston, M. A. & Wolverton, S. (2009). The global distribution of net primary production: resolving the paradox. *Ecological Monographs* (*79*)3 (Aug., 2009), pp. 343-377.

Ishwaran, H., & Kogalur, U. B. (2021). Fast Unified Random Forests for Survival, Regression, and Classification (RF-SRC) (R package version 2.10.1). https://cran.r-project.org/package=randomForestSRC

Jha, C.S. & J.S. Singh. (1990). Composition and dynamics of dry tropical forest in relation to soil texture. *Journal of Vegetational Science 1,* 609–614.

Jones, C.G., & Lawton, J.H. (1995). Linking Species and Ecosystems: Organisms as Ecosystem Engineers. In: Jones C.G., Lawton J.H. (eds) *Linking Species & Ecosystems*. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-1773-3_14

Kaluthota, S., Pearce, D. W., Evans, L. M., Letts, M. G., Whitham, T. G., & Rood, S. B. (2015). Higher photosynthetic capacity from higher latitude: Foliar characteristics and gas exchange of southern, central and northern populations of Populus angustifolia. *Tree Physiology, 35*(9), 936–948. https://doi.org/10.1093/treephys/tpv069

Kawahara, T., Kanazawa, Y. & Sakural, K. (1981). Biomass and net production of man-made forests in the Philippines. *Journal of the Japanese Forestry Society. 63*(9): 320–327.

Kijowska-Oberc, J., Staszak, A. M., & Ratajczak, E. (2020). Adaptation of Forest Trees to Rapidly Changing Climate. *Forests, 11*(123), 1–23. https://doi.org/10.3390/f11020123

Krijthe, J. H. (2015). Rtsne: T-Distributed Stochastic Neighbor Embedding using a Barnes-Hut Implementation.

Kuhn, M. (2020). caret: Classification and Regression Training (R package version 6.0-86). https://cran.r-project.org/package=caretref

Landsberg, J. J., Waring, R. H., & Williams, M. (2020). The assessment of NPP / GPP ratio. *Tree Physiology, 40,* 695–699. https://doi.org/10.1093/treephys/tpaa016

Langner, A., Miettinen, J. & Siegert, F. (2007) Land cover change 2002-2005 in Borneo and the role of fire derived from MODIS imagery. *Global Change Biology, 13*, 2329–2340.

Laurance, W.F. & Fearnside, P.M. (1999). Amazon burning. *Trends in Ecology and Evolution, 14*, 457.

Laurans, M., Martin, O., Nicolini, E., & Vincent, G. (2012). Functional traits and their plasticity predict tropical trees regeneration niche even among species with intermediate light requirements. Journal of Ecology, 100(6), 1440–1452. https://doi.org/10.1111/j.1365-2745.2012.02007.x

Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: Revisiting the Holy Grail. *Functional Ecology, 16*(5), 545–556. https://doi.org/10.1046/j.1365-2435.2002.00664.x

Ledo, A., Paul, K.A., Burslem, D.F.R.P., Ewel, J.J., Barton, C., Battaglia, M., Brooksbank, K., Carter, J., Haakon Eid, T., England, J.R., Fitzgerald, A., Jonshon, J., Mencuccini, M., Montagu, K.D., Montero, G., Ancelm Jugasha, W., Pinkard, E., Roxburgh, S., Ryan, C.M....Chave, J. (2017). Tree size and climatic water deficit control root to shoot ratio in individual trees globally. *New Phytologist 217* (1) 8-11. 2017. https://doi.org/10.1111/nph.14863

Lemon, S. C., Ph, D., Roy, J., Ph, D., Clark, M. A., Ph, D., Rakowski, W., Ph, D., & Med, A. B. (2003). Classification and Regression Tree Analysis in Public Health: Methodological Review and Comparison With Logistic Regression. *The Society of Behavioral Medicine,* 172–181.

Letts, M.G., Rodríguez-Calcerrada, J., & Rolo, V. (2012). Long-term physiological and morphological acclimation by the evergreen shrub *Buxus sempervirens* L. to understory and canopy gap light intensities. *Trees 26*, 479–491 (2012). https://doi.org/10.1007/s00468-011-0609-z

Lewontin, R. C., & Cohen, D. (1969). On population growth in a randomly varying environment. *Proceedings of the National Academy of Sciences of the United States of America, 62*(4), 1056–1060. https://doi.org/10.1073/pnas.62.4.1056

Liaw, A., & Wiene, M. (2002). Classification and Regression by randomForest.

Lilleskov, E.A., Wargo, P.M., Vogt, K.A., Vogt, D.J. (2008). Mycorrhizal fungal community relationship to root nitrogen concentration over a regional atmospheric nitrogen deposition gradient in the northeastern USA. *Can J For Res 38*: 1260-1266.

Litton, C.M., Raich, J.A., & Ryan, M.G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology 13*: 2089- 2109.

Luyssaert, S., Inglima, I., Jung, M., Richardson, A. D., Reichstein, M., Papal, D., Piao, S. L., Schulze, E-D., Wingate, L., Matteucci,G., Aragao, L., Aubinet, M., Beer, C., Bernhofer, C., Black, K. G., Bonal, D., Bonnefond, J-M., Chambers, J., Ciais, P...Janssens, I. A. (2007). CO2 balance of boreal, temperate, and tropical forests derived from a global database. Global Change Biology 13: 2509–2537.

Maaten, L. Van Der, & Hinton, G. (2008). Visualizing Data using t-SNE. *Journal of Machine Learning Research, 9,* 2579–2605.

Mantero, A., & Ishwaran, H. (2021). Unsupervised random forests. *Statistical Analysis and Data Mining, 14*(2), 144–167. https://doi.org/10.1002/sam.11498

Marchand, P., Comita, L. S., Wright, S. J., Condit, R., Hubbell, S. P., & Beckman, N. G. (2020). Seed-to-seedling transitions exhibit distance-dependent mortality but no strong spacing effects in a Neotropical forest. Ecology, (101)2. https://doi.org/10.1002/ecy.2926

Maron, P.-A., Mougel, C., & Ranjard, L. (2011). Soil microbial diversity: Methodological strategy, spatial overview and functional interest. *Comptes Rendus Biologies, 334*(5–6), 403–411. https://doi.org/10.1016/j.crvi.2010.12.003

Moran, N. (1992). The Evolutionary Maintenance of Alternative Phenotypes. *The American Naturalist, 139*(5), 971-989. Retrieved June 18, 2021, from http://www.jstor.org/stable/2462363

NASA. 2013. Study finds severe climate jeopardizing Amazon Forest. 01.17.13. http://www.nasa.gov/topics/earth/features/earth20130117.html, accessed 17 January 2013.

Northrup, R.R., Yu, Z., Dahlgren, R.A., & Vogt, K.A. (1995). Polyphenol control of nitrogen release from pine litter. *Nature 377:* 227-229.

Oostra, V., Saastamoinen, M., Zwaan, B. J., & Wheat, C. W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications, 9*(1). https://doi.org/10.1038/s41467-018-03384-9

O'Neill, R.V., Deangelis, D.L., Waide, J.B., & Allen, T.F.H. (1986). A Hierarchical Concept of Ecosystems. *Monographs in Population Biology, No. 23*. Princeton, NJ: Princeton University Press. ISBN 069108436XOCLC13526197.

Pagel, J., Treurnicht, M., Bond, W. J., Kraaij, T., Nottebrock, H., Schutte-Vlok, A. L., Tonnabel, J., Esler, K. J., & Schurr, F. M. (2020). Mismatches between demographic niches and geographic distributions are strongest in poorly dispersed and highly persistent plant species. *Proceedings of the National Academy of Sciences of the United States of America, 117*(7), 3663–3669. https://doi.org/10.1073/pnas.1908684117

Palmiotto, P.A., Davies, S.J., Vogt, K.A., Ashton, P.M.S., Vogt, D.J., & Ashton, A.S. (2004). Soil-related habitat specialization in dipterocarp rain forest tree species in Borneo. *J of Ecol. 92*: 609-623.

Paoli, G.D., & Curran, L.M. (2007). Soil Nutrients Limit Fine Litter Production and Tree Growth in Mature Lowland Forest of Southwestern Borneo. *Ecosystems. Vol. 10,* 503–518.

Parrotta, J. A., Knowles, O. H., & Wunderle, J. M. (1997). Development of floristic diversity in 10-year-old restoration forests on a bauxite mined site in Amazonia. *Forest Ecology and Management, 99*(1-2):21-42.

Peacock, K. A. (2011). The three faces of ecological fitness. Studies in History and Philosophy of Science Part C: *Studies in History and Philosophy of Biological and Biomedical Sciences, 42*(1), 99–105. https://doi.org/10.1016/j.shpsc.2010.11.011

Pigliucci, M., Murren, C. J., & Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *The Journal of Experimental Biology, 209,* 2362–2367. https://doi.org/10.1242/jeb.02070

Potter, C.S., Davidson, E.A., Klooster, S.A., Nepstad, D.C., de Negreiros, G.H., & Brooks, V. (1998). Regional application of an ecosystem production model for studies of biogeochemistry in Brazilian Amazonia. *Global Change Biology, 4* (3), 315}334.

Potts, M.D. (2003). Drought in a Bornean everwet rain forest. *J of Ecology 91*: 467-474.

Proctor, J., Lee, Y. F., Langley, A. M., Munro, W. R. C., & Nelson, T. (1988). Ecological Studies on Gunung Silam, A Small Ultrabasic Mountain in Sabah, Malaysia. I. Environment, Forest Structure and Floristics. *Journal of Ecology 76* (2) 320-40. https://doi.org/10.2307/2260596.

Propastin P. (2011) Release of Carbon Dioxide by Terrestrial Ecosystems Over Indonesia During the 1997–1998 ENSO Warm Event. In: Leal Filho W. (eds) The Economic, Social and Political Elements of Climate Change. *Climate Change Management*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-14776-0_20.

Qi, Y., Wei, W., Chen, C., & Chen, L. (2019). Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecology and Conservation. Vol 18*, 1-14. April 2019. https://doi.org/10.1016/j.gecco.2019.e00606.

Raich, J.W., & Nadelhoffer, K.J. (1989). Belowground carbon allocation in forest ecosystems: global trends. *Ecology 70:* 1346-1354.

Reich, P.B., Walters, M.B., & Ellsworth, D.S. (1992). Leaf lifespan in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecological Monographs 62:* 365–392.

Rieseberg, L.H., Widmer, A., Arntz, A.M., & Burke, J.M. (2002). Directional selection is the primary cause of phenotypic diversification. *Poceedings National Acad Science 99*(19), 12242-12245. doi:10.1073/pnas.192360899.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

Genuer, R., Poggi, J.-M., & Tuleau-Malot, C. (2019). VSURF: Variable Selection Using Random Forests. R package version 1.1.0. https://CRAN.R-project.org/package=VSURF

Rousk, J., Brookes, P. C., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology, 75*(6), 1589–1596. https://doi.org/10.1128/AEM.02775-08

Running, S.W., & Zhao, M., (2015). User's Guide. Daily GPP and Annual NPP (MOD17A2/A3) Products NASA Earth Observing System MODIS Land Algorithm. Version 3.0 For Collection 6. October 7, 2015.

Ryan, M.G. (1991). A simple method for estimating gross carbon budgets for vegetation in forest ecosystems. *Tree Physiology 9*: 255-266.

Sánchez-Gómez, D., Valladares, F., & Zavala, M. A. (2006). Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species. *Tree Physiology, 26*, 1425– 1433.

Scheffer, M., Hirota, M., Holmgren, M., Nes, E.H.V., & Chapin, F.S. (2012). Thresholds for boreal biome transitions. *Proceedings of the National Academy of Sciences USA. 109*:21384–21389.

Scheffer, M., Barrett, S., Carpenter, S.R., Folke, C., Green, A.J., Holmgren, M., Hughes, T.P., Kosten, S., van de Leemput, I.A., Nepstad, D.C., van Nes, E.H., Peeters, E.T.H.M. & Walker, B. (2015). Creating a safe operating space for ecosystems. *Science 347* (6228), 1317-1319.

Schlichting, C. D. & Pigliucci, M. (1998). Phenotypic Evolution: A Reaction Norm Perspective. Sunderland, MA: Sinauer Associates.

Shivaprakash, K. N., Ramesh, B. R., Umashaanker, R., & Dayanandan, S. (2018). Functional trait and community phylogenetic analyses reveal environmental filtering as the major determinant of assembly of tropical forest tree communities in the Western Ghats biodiversity hotspot in India. *Forest Ecosystems, 5*(25), 1–16.

Shugart, H.H. (1984). A Theory of Forest Dynamics. *The Ecological Implications of Forest Succession Models.* Springer-Verlag. New York.

Sigurdardottir, R.R., Vogt, K. A. & Vogt, D. J. (1999). *Effects of different forest types on soil leachates in eastern Iceland.* In Geochemistry of the Earth's Surface. (pp. 397-402). A.A. Balkema.

Smith, F. C. (1993). Evergreen vs . Deciduous Woody Plants: Which Wins Where Author (s): Frances C . Smith Published by : Eagle Hill Institute Stable URL : https://www.jstor.org/stable/3858181. *Maine Naturalists, 1*(4), 205–212.

Smith, S.E., & Read, D. (2008). Mycorrhizal Symbiosis. 3rd ed. Elsevier Ltd.

Song, X., Hansen, M.C., Stehman, S.V., Potapov, P.V., Tyukavina, A., Vermote, E.F., & Townshend, J.R. (2018). Global land change from 1982 to 2016. *Nature 560*, 639–643. https://doi.org/10.1038/s41586-018-0411-9.

Strobl, C., Boulesteix, A. L., Kneib, T., Augustin, T., & Zeileis, A. (2008). Conditional variable importance for random forests. *BMC Bioinformatics, 9*, 1–11. https://doi.org/10.1186/1471-2105-9-307

Strobl, C., Hothorn, T., & Zeileis, A. (2009). Party on! *R Journal, 1*(2), 14–17. https://doi.org/10.32614/rj-2009-013

Sprugel, D. G. (1989). The relationship of evergreenness, crown architecture, and leaf size. *American Naturalist 133:* 465–479.

Sprugel, D.G., Ryan, M.K., Brooks, J.R., Vogt, K.A., & Martin, T.A. (1994). Respiration from the organ-level to the stand-a model system for the application of scaling techniques. In: pp. 255-299. (W.K. Smith and T.M. Hinckley, eds.) *Resource Physiology of Conifers. Acquisition, Allocation, and Utilization.* Academic Press. San Diego, New York.

Taylor, P.G., Cleveland, C.C., Soper, F., Wieder, W.R., Dobrowski, S.Z., Doughty, C.E., & Townsend, A.R. (2019). Greater stem, growth, woody allocation, and aboveground biomass in Paleotropical forests than in Neotropical forests. *Ecology 100*(3). https://doi.org/10.1002/ecy.2589

Tchokponhoué, D. A., Danikou, S. N., Houéto, J. S., & Achigan-dako, E. G. (2019). Shade and nutrient-mediated phenotypic plasticity in the miracle plant Synsepalum dulcificum. *Nature Scientific Reports, May 2018, 1*–11. https://doi.org/10.1038/s41598-019-41673-5

Temesgen, H., Affleck, D., Poudel, K., Gray, A., & Sessions, J. (2015). A review of the challenges and opportunities in estimating above ground forest biomass using tree-level models. *Scandinavian J of Forest Research 30* (4): 326-335. https://doi.org/10.1080/02827581.2015.1012114

Thompson, K. & Grime, J. P. (1983). A Comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology, (20).*

Tonin, A. M., Gonçalves, J. F. Jr., Bambi, P., Sheyla, R., Couceiro, M., Lorrane A., Feitoza, M., Fontana, L. E., Hamada, N., Hepp, L. U., Lezan-Kowalczuk, V. G., Leite, G. F. M., Lemes-Silva, A. L., Lisboa, L. K., Loureiro, R. C., Martins, R. T., & Boyero, L. (2017). Plant litter dynamics in the forest-stream interface: precipitation is a major control across tropical biomes. *Sci Rep 7* , 10799 (2017). https://doi.org/10.1038/s41598-017- 10576-8.

Torsten Hothorn, & Zeileis, A. (2015). partykit: A Modular Toolkit for Recursive Partytioning in R (pp. 3905–3909).

Torsten, H., & Zeileis, A. (2015). partykit: A Modular Toolkit for Recursive Partytioning in R. *Journal of Machine Learning Research, 16*, 3905-3909. URL https://jmlr.org/papers/v16/hothorn15a.html

Trappe, J.M., & Luoma, D.L. (1992). *The ties that bind: Fungi in ecosystems. Ch2: Pp. 17-27*. The Fungal Community. Its Organization and Role in the Ecosystems. Eds. GC Carroll, DT Wicklow. Marcel Dekker, Inc. New York.

Trouillier, M., Maaten-theunissen, M. Van Der, & Scharnweber, T. (2020). A Unifying Concept for Growth Trends of Trees and Forests – The "Potential Natural Forest." *Frontiers in Forest and Global Change, 3(September)*. https://doi.org/10.3389/ffgc.2020.581334

Tufto, J. (2000). The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *American Naturalist, 156*(2), 121–130. https://doi.org/10.1086/303381

USDA Soil Taxonomy. (1999). Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. 2nd edition. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook 436.

Valladares, F., Sanchez-Gomez, D., & Zavala, M. A. (2006). Quantitative estimation of phenotypic plasticity: Bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology, 94*(6), 1103–1116. https://doi.org/10.1111/j.1365-2745.2006.01176.x

Van Tienderen, P. H. (1991). Evolution of Generalists and Specialists in Spatially. *Evolution, 45*(6), 1317–1331.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlichting, C.D., & Van Tienderen, P.H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol Evol. 10*(5):212-7. doi: 10.1016/s0169-5347(00)89061-8. PMID: 21237012.

Violle, C., Navas, M., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., Oikos, S., May, N., Violle, C., Navas, M., Vile, D., Kazakou, E., & Fortunel, C. (2007). Let the concept of trait be functional! *Oikos, 116*(5), 882–892.

Vogt, K.A., Grier, C.C., Meier, C.E., & Edmonds, R.L. (1982). Mycorrhizal role in net primary production and nutrient cycling in Abies amabilis ecosystems in western Washington. *Ecology 63*(2): 370-380.

Vogt, K.A., Grier, C.C., Vogt, D.J. (1986). Production, turnover, and nutrient dynamics of above- and belowground detritus of world forests. *Advances in Ecol. Res. 15*: 303-377.

Vogt, K. A. (1991). Carbon budgets of temperate forest ecosystems. *Tree Physiol. Jul-Sep 1991;9*(1_2):69-86. DOI: 10.1093/treephys/9.1-2.69.

Vogt, K.A., Publicover, D.A., Bloomfield, J., Perez, J.M., Vogt, D.J., & Silver, W.L. (1993). Belowground responses as indicators of environmental change. *Environmental and Experimental Botany 33*(1): 189-205.

Vogt, K. A., Vogt, D. J., Asbjornsen, H., & Dahlgren, R. A. (1995). Roots, nutrients and their relationship to spatial patterns. *Plant and Soil, 168–169*(1), 113–123. https://doi.org/10.1007/BF00029320

Vogt, K.A., Asbjornsen, H., Ercelawn, A., Montagnini, F., & Valdes, M., (1997). Ecosystem integration of roots and mycorrhizas in plantations. Pp. 247-296. In: S. Nambiar, A. Brown, eds. *Management of Soil, Water and Nutrients in Tropical Plantation Forests*. ACIAR. Australia.

Vogt KA, J Bloomfield. (1991). *Root turnover and senescence. pp. 287-306*. In: (Y. Waisel, A. Eshel and U. Kafkafi, eds.) Plant Roots: the Hidden Half. Marcel Dekker, Inc. New York, Basel.

Vogt, K.A., Publicover, D. A., Bloomfield, J., Perez, J. M., Vogt, D. J., & Silver, W. L. (1993). Belowground responses as indicators of environmental change. *Environmental and Experimental Botany 33*(1): 189-205.

Vogt KA, DJ Vogt, J Bloomfield. 1998. Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant and Soil 200: 71-89.

Vogt KA, H Asbjornsen, A Ercelawn, F Montagnini, M Valdes. 1997. Ecosystem integration of roots and mycorrhizas in plantations. Pp. 247-296. In: S. Nambiar, A. Brown, eds. Management of Soil, Water and Nutrients in Tropical Plantation Forests. ACIAR. Australia.

Vogt, K.A, J. Gordon, J. Wargo, D. Vogt, H. Asbjornsen, P.A. Palmiotto, H. Clark, J. O'Hara, W.S. Keeton, T. Patel-Weynand, E. Witten with contributions by B Larson, D Tortoriello, J Perez, A Marsh, M Corbett, K Kaneda, F Meyerson, D Smith. (1997). Ecosystems: Balancing Science with Management. Springer-Verlag. NY. Pp. 470.

Waring, R. H. (1986). *Comparison of Methods of Estimating Leaf-Area Index*. In Old-Growth Douglas-Fir. The Ecological Society of America, Washington, D.C. (67)4, pp. 975-979.

Waring, R. H. (1987). Characteristics of trees predisposed to die. *BioScience 37*(8), 569-574. https://doi.org/10.2307/1310667.

Waring, R. H., Landsberg, J. J., & Williams, M. (1998). Net primary production of forests: a constant fraction of gross primary production? *Tree Physiology, 18*(1998), 129–134.

Waring, R. H., & Franklin, J. F. (1979). Evergreen coniferous forests of the Pacific Northwest. *Science, 204*:1380-1386.pdf.

Wu, J., van der Linden, L., Lasslop, G., Carvalhais, N., Pilegaard, K., Beier, C., and Ibrom, A.: Effects of climate variability and functional changes on the interannual variation of the carbon balance in a temperate deciduous forest, Biogeosciences, 9, 13–28, https://doi.org/10.5194/bg-9-13-2012, 2012.

Xu, Q., Man, A., Fredrickson, M., Hou, Z., Pitkänen, J., Wing, B., Ramirez, C., Li, B., & Greenberg, J.A. (2018). Quantification of uncertainty in aboveground biomass estimates derived from small-footprint airborne LiDAR. *Remote Sensing of Environment 216*: 514-528.

Yuan, Z.Y., & Chen, H.Y.H. (2010). Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility and stand age: Literature review and meta- analyses. *Critical Reviews in Plant Sciences 29*: 204-221.

Zhang, G., Zhang, L., & Wen, D. (2018). Photosynthesis of subtropical forest species from different successional status in relation to foliar nutrients and phosphorus fractions. *Scientific reports 8*, 10455 (2018). https://doi.org/10.1038/s41598-018-28800-4.

Zheng, M., Jagota, A., Semke, E. D., Diner, B. A., McLean, R. S., Lustig, S. R., Richardson, R. E., & Tassi, N. G. (2003). DNA-assisted dispersion and separation of carbon nanotubes. *Nat Mater. May;2*(5):338-42. doi: 10.1038/nmat877. PMID: 12692536.

Chapter 5 : Toward a Synthesis of Ecological Assessment

Humans are biological organisms requiring the same natural resources as elephants, bark beetles, and cyanobacteria; at its most basic, they require a suitable habitat, a source of energy and nutrients, and symbiotic relationships with other organisms. Human beings require natural resources not only to meet our basic biological needs but also to meet our socio-economic needs. The human enterprise has accelerated exponentially over the last millennium due in large part to advances in agriculture, management of injury and disease, a structured form of governance, formalized education, and development and use of technologies (Vitousek et al., 1997). To meet the demands of our highly technological society we have disproportionately co-opted natural resources which has directly lowered the carrying capacity for all other life forms (except for some generalist species) (Cardinale et al., 2002).

Modernity has exhausted many natural resources, transformed 75% of all vegetated lands and divvied up what remains to the detriment of ecosystems at the global scale (IPBES, 2019). There is a growing concern that humanity is already outside its safe operating space in terms of biogeochemical cycles (excessive nutrient loading to ecosystems), biodiversity loss, and climate and very close to other critical limits (Fig. 5-1) (Steffen et al., 2015).

"Ecosystems, species, wild populations, local varieties and breeds of domesticated plants and animals are shrinking, deteriorating or vanishing. The essential, interconnected web of life on Earth is getting smaller and increasingly frayed. This loss is a direct result of human activity and constitutes a direct threat to human well-being in all regions of the world."

[Josef Settele, IPBES, 2019]

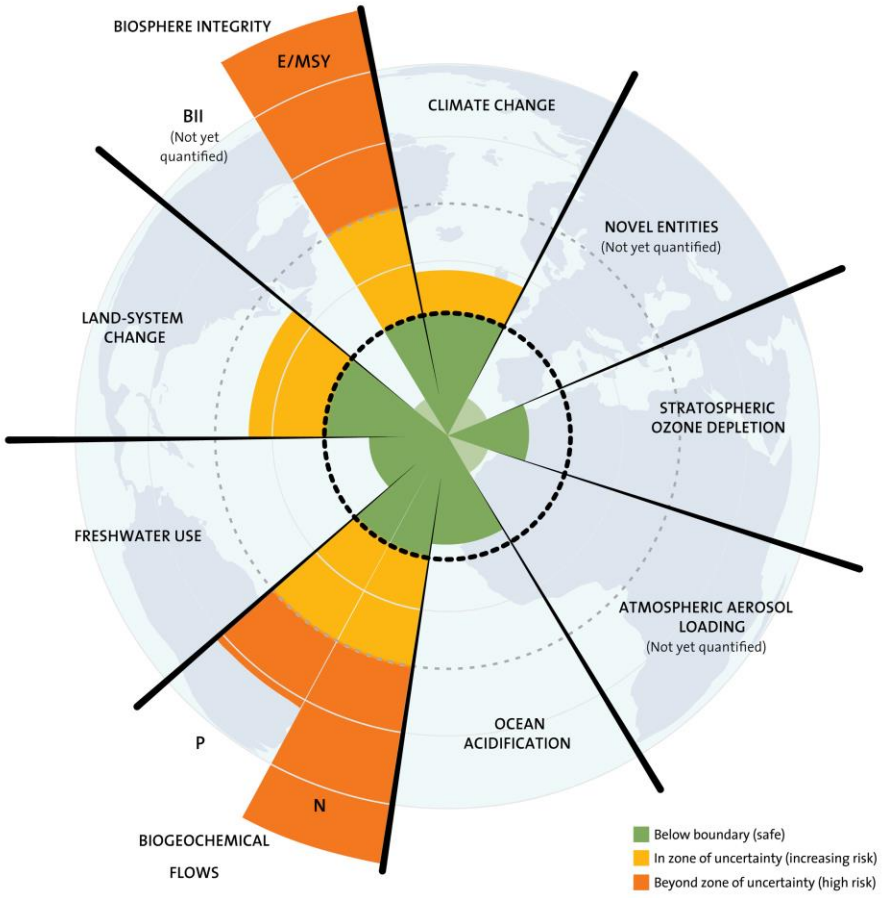


Figure 5-1: There are three critical planetary boundaries at high risk. Severe interruptions to flows of N and P, extinction rate and extinctions per million species per year (E/MSY) affecting ecosystem functioning at continental and ocean basin scales. Impact on many other boundaries—C storage, freshwater, N and P cycles, land systems. https://www.stockholmresilience.org/research/planetary-boundaries.html (J. Lokrantz/Azote based on Steffen et al.. 2015).

The ability of humanity to respect planetary boundaries will depend on how well we can decouple our socio-economic need for growth from our exhaustive demand for resources. The extractive industries driven by consumerism and intensive agriculture has polluted air and water and turned living soils into unproductive wastelands. The knock-on effects are major disruptions to ecosystem function across all domains, the magnitude of which are yet to be fully realized. With rare

exception, altered nutrient and water cycles have resulted in declines in ecological productivity across all trophic compartments in every biome. Combined, exhausted freshwater aquifers, collapsed fisheries and a globally destabilized climate, focus us on how the human endeavor threatens the very habitability of the planet. Humans depend on the productivity of the earths' ecosystems directly for natural resources and services, but what is ecological productivity, how do we measure it and by what metric do we assign it value? How well does our current framing of ecological paradigms capture its true importance to humans and the persistence of the natural world?

Ecosystems vary in size, function and complexity and are dynamic as conditions change and the unique periodicity of the climate and symbiotic relationships wax and wane over time. The ability of any species to respond to disturbance is based upon the traits and symbioses that evolved over millennia to the unique consortia of conditions that occurred in the past. These traits allowed species to thrive so long as conditions remained within the normal range of a species tolerance.

The geological and climatic context are first-order determinants of biome characteristics and ecosystem properties at large spatial scales, which determine population distribution patterns within communities. At smaller scales, different combinations of environmental conditions and ecological relationships determine the level of productivity of populations. At the scale of the organism, life-history, symbiotic relationships, and trait plasticity are determinative of individual productivity with epigenetic factors modulated by the environment playing a significant role in adaptive capacity. At the foundation of ecological productivity, biogeochemical cycles modulated by microbial metabolism, and their interactions via by-product food webs, function as bottle-up constraints to nutrient stoichiometry. Given that the climate is significantly changing, and temperature increases are "baked-in" (IPCC, 2007) we must incorporate the preeminence of microbiology and a whole systems approach to natural ecology to save ourselves (Fig. 5-2).

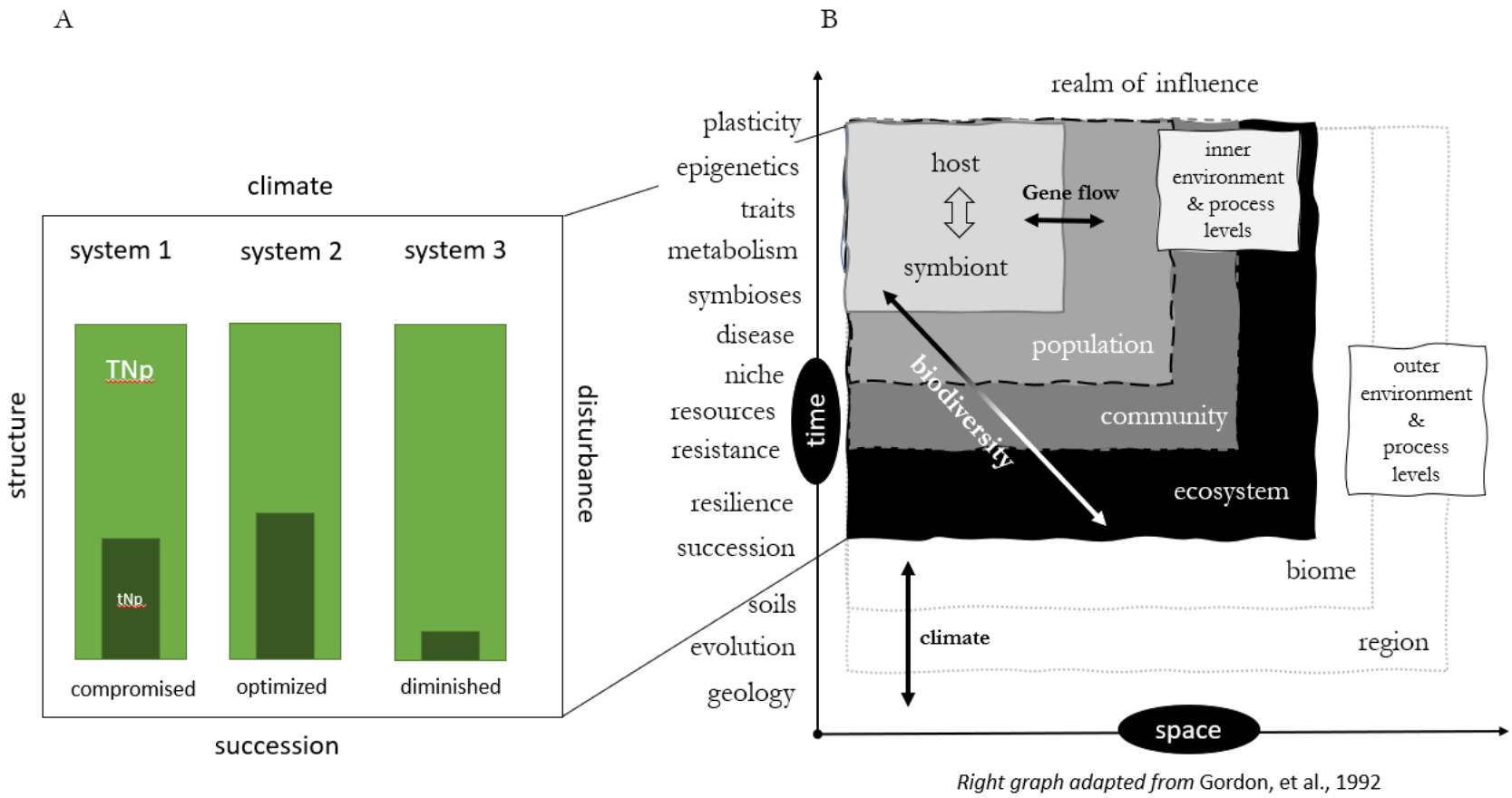


Figure 5-2: (B) Ecosystems exhibit emergent properties within their geological, evolutionary, and climatic contexts. Soil development and successional change determine ecosystem resilience to disturbance over time and is characterized as the influence of disturbance on subsequent structure and composition of biological communities. Ecological resistance is how community structure and composition tolerate different types of disturbance, dependent upon available resources, biodiversity, and level of niche partitioning. Looking inside the "black box" of ecological models, this framework accounts for the impact of disease, symbiotic relationships, and the collective metabolism of the holobiont. Each individual has unique genetic traits, epigenetic factors, and phenotypic plasticity that controls the species' adaptive capacity that promulgates to higher levels of organization. (A) We use the ratio of measured total net productivity (tNp) to the internal reference of the theoretical maximum productivity (TNp), i.e.,

“ecosystem fit” for primary productivity, or “adaptive fit” for secondary productivity. Low productivity is not necessarily a sign of system failure, but comparisons across systems with similar successional stage, community and environmental structure, climate regime, and level of disturbance allows evaluation of how far away the system may be from a threshold or system state-change.

Ecology has operated with heuristically frugal approaches in the past, and it is hard to break the pattern, but we must expand our concept of how ecosystems function as emergent to all the biotic and abiotic components with special attention to the closest symbiotic interactions. A common definition of ecological function is productivity measured as biomass. To incorporate the inherent complexities in measuring biomass, I developed a holistic ecological framework that expands our current theoretical paradigm of productivity to promote the central importance of microbiology in a richer and more realistic biological context. I have attempted to reconcile the dominant eco-physiological processes of primary and secondary productivity using well-established metrics and methods. I conclude that an interpretation based on holistic ecological principles that incorporates species and processes, operating at different spatial and ecological scales, across different trophic compartments, will more effectively couple the complex processes of natural ecophysiology to changing environmental conditions.

I envision the "productivity landscape" as a conceptual tool to accomplish this, to question both the lay public assumption and willful cognitive dissonance of the intelligentsia, that ecosystems are stable and will remain so across space and time no matter the pressure and damage society imposes. I also challenge the complex and highly derived computer models that overly rely on environmental forcing to understand ecosystem behavior. The foundational disciplines of ecology are population ecology, community ecology, ecosystem ecology, and evolutionary ecology. A new integrated, real-world approach is required that reflects how biodiversity and ecological function are coupled (Loreau, 2010). Ecological productivity, biodiversity, and resilience are "joined at the hip," and we must integrate the dynamics of the climate, environmental disturbance, community structure and function, and the physiology of symbioses between organisms. The "trait space" of the holobiont accounts for all the host-symbiont phenotypic traits and the plasticity of those traits

within a system at any scale. Ecosystems exists across a range of productivity to a range of resilience, dependent upon the adaptive capacity of the trait space that flows from the genetic limitations of each holobiont, each population, and each community. The relative contribution of each holobiont species will flow from its ranking as a keystone species within its ecosystem. This model tells us that ecosystem productivity is not related to resilience as some systems are less productive due to internal and/or external constraints. Therefore, ecosystems with high productivity can have low resilience, i.e., coral reefs, and ecosystems with low productivity can have high resilience, i.e., deep-marine vents. Although we must always account for the significance of climate, we must simultaneously account for small scale modifiers, community complexity (i.e., biodiversity, food web length), ecological relationships (i.e., holobiont [host-symbionts], parasitism, herbivory, predation, and pathogens), the impact of invasive species, pollution, as well as the increasing frequency and magnitude of stochastic disturbance within the long-term trend of climate change (Isbell et al., 2009). Life functions in the variance and survives in the margins of its safe operating space. Each trophic compartment, in each ecosystem with its respective organisms, displays a unique set of dynamics, level of specialization, and phenotypic plasticity that may confer advantages to some species whereas others will go extinct gradually over evolutionary time or quickly in the face of rapid environmental change. If we better understand the proclivities of each of these compartments, within the context of its ecosystem, we may be able to better manage the decline of biodiversity and stave off, at least temporarily, the worst consequences of the Anthropocene for our children and grandchildren.

References

Cardinale, B. J., Palmer, M. A., & Collins, S. L. (2002). Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature 415*, 426–429.

Gordon, J.C., Bormann, B.T., & Kiester, A.R. (1992). The physiology and genetics of ecosystems: A new target or "Forestry contemplates an entangled bank". *Proceedings of the 12th North American Forest Biology Workshop*. Sault Ste. Marie, Ontario, Canada. Aug 17-20, 1992. Ontario Ministry of Natural Resources, Ontario Forest Research Institute and Forestry Canada, Ontario Region, pp. 1-14.

Intergovernmental Panel on Climate Change (IPCC). (2007). *AR6 Climate Change 2022: Impacts, Adaptation and Vulnerability*. Geneva.

IPBES. (2019). *IPBES Global Assessment Summary for Policymakers*. https://ipbes.net/system/tdf/ipbes_global_assessment_report_summary_for_policymakers.pdf?file=1&type=node&id=35329

Isbell, F. I., Polley, H. W., & Wilsey, B. J. (2009). Biodiversity, productivity, and the temporal stability of productivity: Patterns and processes. *Ecology Letters 12,* 443–451.

Loreau, M. (2010). From *Populations to Ecosystems: Theoretical Foundations for a New Ecological Synthesis.* Princeton, NJ: Princeton University Press.

Marris, E. (2010). UN body will assess ecosystems and biodiversity. *Nature 465*, 859.

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., De Vries, W., De Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B., & Sörlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science, 347*(6223). https://doi.org/10.1126/science.1259855

United Nations. (2021). Sustainable Development Goals. https://www.un.org/sustainabledevelopment/blog/2019/05/nature-decline-unprecedented-report/

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science, 277*(5325), 494–499. https://doi.org/10.1126/science.277.5325.494