Biodiversity and Ecosystem Functioning

What Diversity? Which Functioning?

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"…when people thought the earth was flat, they were wrong. When people thought the earth was spherical, they were wrong. But if you think that thinking the earth is spherical is just as wrong as thinking the earth is flat, then your view is wronger than both of them put together."

Isaac Asimov

Abstract

We share our planet with an estimated 8.7 million eukaryotic species and an uncountable number of bacteria and archaea. But that amazing diversity is under threat from overexploitation, habitat destruction and climate change. This realization has lead ecologists to study the consequences of species loss. The consensus after 30 years of research is that biodiversity can have many benefits. More diverse communities tend to be more productive and more stable. But the research has mostly focused on diversity at the level of species, in relatively species-poor ecosystems, and often measured diversity as the number of species - independent of their identity or relative abundance. In this thesis I leverage the advancements of modern sequencing technology to use mega-diverse bacterial communities as a model system. The thesis includes four chapters.

Chapter I shows that bacterial freshwater communities sustain ecosystem functioning despite extensive reductions in diversity. A literature review corroborates the results - only 25 % of the reported experimental manipulations show a positive effect of bacterial diversity on ecosystem functioning.

In **Chapter II**, we investigate the effects of habitat diversity on ecosystem functioning. We use experimental landscapes of shallow bay sediment habitats. Depending on the season, both greater habitat diversity and greater bacterial diversity increase landscape ecosystem functioning.

Chapter III, in which we relate the diversity of microbial denitrifiers to nitrogen fixation rates in natural marine sediments, shows no connection between diversity and functioning. Nor can other microbial community metrics be related to nitrogen fixation rates, including the diversity of the general bacterial community and the abundance of certain species. In a previous study, nitrogen fixation correlated to the abundance of the genes that encode the protein involved in the process (*nifH* genes). Yet, that model fails to predict nitrogen fixation rates in our study.

Chapter IV is about the "functioning" part in biodiversity and ecosystem functioning research. It has been suggested, that while biodiversity is only weakly important for single functions, its importance increases when

multiple functions are considered simultaneously. The logic is intuitively appealing: if species perform different functions, more species are needed to perform more functions. Nonetheless, it is wrong. We show that considering multiple functions does not per se change the biodiversity-ecosystem functioning relationship.

In concert, the four chapters included in this thesis call into question some of the broad claims that have been made in the field of biodiversity and ecosystem functioning. The number of species as such is unlikely to be generally related to ecosystem functioning, especially in highly diverse systems. Claims that any species loss will result in loss of ecosystem functioning cannot be justified. Jointly considering multiple functions does not change that conclusion. Nevertheless, protecting diversity is a moral imperative, and inflicting irreversible changes to nature without understanding the consequences is careless and shortsighted. As human impact is unavoidable, we need the best possible knowledge base to make evidence- based and informed decisions. Research in ecology is crucial to provide this knowledge. To be reliable it must be as rigorous as possible. This thesis hopes to provide some small steps in the right direction.

Sammanfattning

Ett av jordens mest unika karaktärsdrag är dess mångfald av liv. Vi människor delar vår planet med omkring 8.7 miljoner arter. Oavsett om vi inser det eller inte är vi beroende av dem. Vi äter mat från havet och våra grödor pollineras av hundratals olika insektsarter. 75% av all cancermedicin är naturliga produkter, liksom 60% av vår antibiotika.

Samtidigt är det mänskliga trycket på naturen idag större än någonsin. Vi finkammar havet med industriella fiskeflottor, och skövlar våra skogar i jakt på virke och pappersmassa. Ungefär 40% av världens landyta används idag för jordbruk. Samtidigt ökar trycket på naturen till följd av klimatförändringar som uppvärmning och havsförsurning. Detta leder till att vi förlorar arter i en alarmerande takt. Dessa insikter ligger till grund för mitt forskningsområde: biodiversitet och ekosystemens funktion. I ljuset av att den biologiska mångfalden nu snabbt utarmas, vilka är konsekvenserna?

Hur kan biodiversitet vara viktig?

Arter har olika krav både gällande miljöförhållanden och resurser. En växt med pålrot (som till exempel tistlar) kan ta upp vatten och näring som är otillgänglig för växter med fibrösa rötter (som till exempel många gräs), medan en art med fibrösa rötter är effektivare att tillgängliggöra sig vatten och näring i jordens toppskikt. En skuggtolerant ormbunke kan frodas under det täta taket av skuggintoleranta träd. Detta kallas nischuppdelning. Nischuppdelning kan förklara varför ett ekosystem med hög biologisk mångfald ofta är mer produktiva än ekosystem med lägre mångfald.

Är biodiversiteten viktig?

Enligt 30 års experimentell forskning är den det. Gräsmarker med hög biodiversitet är mer produktiva än gräsmarker med färre arter, och varierar mindre mellan år. Samma sak har man funnit i marina ekosystem, i skogar och även i experiment med bakterier. Konsensus är att ekosystemets funktion ökar med ökad biodiversitet.

Men vad menar vi med biodiversitet och ekosystemfunktioner?

Majoriteten av de studier som undersökt betydelsen av biologisk mångfald har studerat artrikedom. Artrikedom betyder helt enkelt endast antalet arter, oavsett vilka de är och om de är vanliga eller sällsynta. Detta är dock en inkomplett bild av vad mångfald är. Föreställ dig en bit skog i höstskrud med tio olika trädslag, alla i ungefär samma antal, och med löv i olika färger. Tänk dig sen en bit planterad granskog, med nio träd av olika arter i utkanten av planteringen. Vilken skog skulle du säga har högst mångfald? Båda har tio arter och ändå skulle de flesta vara överens om att den förra har högre biodiversitet än den senare. Det är heller inte bara arters relativa abundans som spelar in. Även om arterna är nära besläktade eller inte kan vara viktigt.

Men vilken betydelse har den biologiska mångfalden? Det stora flertalet av alla de hundratals experiment som gjorts har inkluderat endast ett fåtal arter, och dessa experiment visar att den största effekten observeras när man går från en till två till tre arter. Men naturliga ekosystem består av hundratals, eller rentav tusentals arter.

Utgångspunkten för denna avhandling var att undersöka de ovannämnda frågorna i naturliga mikrobiella system. Bakteriella system är överväldigande mångfaldiga. Ett gram jord innehåller till exempel mer bakterier än det finns människor på jorden. Dessa bakterier utgörs av tiotusentals olika arter. Att studera en sådan mångfald är utmanande. Vi kan inte skilja mer än en handfull olika former när vi studerar bakterier i mikroskop. Därför måste vi titta på deras gensekvenser för att identifiera dem. Varje organism har en unik DNA-sammansättning. Om vi känner till en gens exakta sekvens, vet vi vilken art den tillhör, och om vi observerar en viss gensekvens för första gången vet vi att vi har hittat en ny art. Dessutom kan vi med hjälp av sekvenser uttala oss om hur nära besläktade olika arter är. Tack vare revolutionerande tekniska framsteg de senaste åren är det idag möjligt att sekvensera och analysera den stora mängd gener som finns i ett mikrobiellt system. Sekvensering kallas den process som ”läser av” den genetiska koden från en bit DNA. Den nya tekniken tillåter oss att sekvensera upp till hundratals miljoner gener parallellt, vilket möjliggör att för första gången noggrant studera mångfalden i system med hög biodiversitet. Sammanfattningsvis kan man säga att bakteriella samhällen utgör ett intressant modellsystem för att studera betydelsen av biologisk mångfald.

I **Kapitel I** i denna avhandling gjorde jag just det. Jag använde mig av bakteriesamhällen från fyra sjöar. Eftersom alla arter inte är lika vanliga använde jag mig av en utspädningsteknik för att utesluta arter från de ursprungliga samhällena. På detta sätt skapade jag en gradient i biodiversitet. De experimentella samhällena placerades utomhus i stora vattenkar och experimentet löpte över sex veckor.

Jag mätte olika aspekter av bakteriesamhällenas mångfald, och tog i beaktande arternas relativa abundans, deras släktskap, och hur funktionellt olika varje samhälle var. Experimentet visade att biodiversitet var av liten betydelse för hur systemets fungerar. Detta var sant oavsett vilket mått på mångfald som användes. En genomgång av de experiment som använt liknande metoder visade att mina resultat inte var unika: endast 25% av experimenten hittade en positiv effekt av biodiversitet. Sammanfattningsvis föreslår resultaten att bakteriella system kan upprätthålla en rad ekosystemfunktioner även om en stor del av arterna försvinner.

I **Kapitel II** var jag och mina kollegor intresserade av effekterna av diversitet på livsmiljönivå. Biologisk mångfald innefattar inte bara arter, utan alla organisationsnivåer, inklusive livsmiljöer. En stor mänsklig påverkan både på land och i havet är en homogenisering av landskapet. Marina mjukbottnar har en hög komplexitet på liten skala, även om denna komplexitet ofta är svår att se med bara ögat. När en bottentrål plogar havsbotten lämnar den efter sig ett homogeniserat landskap. Samma sak sker på land. Genom att konvertera stora områden för jordbruk gör vi dem alla lika. Vi minskar biodiversiteten av livsmiljöer. I ljuset av detta är det olyckligt att effekterna av en homogenisering av våra landskap på ekosystemens funktion är i stor sett outforskade.

Vi föreslår i **Kapitel II** att olika livsmiljöer kan påverka varandra positivt, precis som arter kan. Tänk exempelvis på samspelet mellan mangrove, sjögräs och korallrev i tropiska kustvatten. Mangroveskogar fångar sediment, som gör vattnet klart, vilket är fördelaktigt för både sjögräs och koraller. Sjögräsängar reducerar också grumligheten och filtrerar näringsämnen från vattnet, vilket begränsar tillväxten av alger på korallreven. Reven ger i sin tur ett fysiskt skydd mot vågor för både sjögräs och mangrove.

I grunda marina vikar längs den svenska västkusten spelar bakterier och mikroskopiska alger en viktig roll för ekosystemets funktion. För dessa organismer representerar olika typer av sediment, som sand eller lera, olika livsmiljöer. Vi satte samman sedimentkärnor från olika livsmiljöer i de grunda vikarna till konstgjorda landskap med varierande diversitet av livsmiljöer (1 - 4 typer). Vi mätte fyra funktioner som drivs av mikroorganismer. Våra resultat visar att landskap med en mångfald av livsmiljöer har högre funktionalitet än landskap med låg mångfald.

Positiva effekter av biodiversitet, som den vi fann i **Kapitel II**, har ofta lett till generella slutsatser att våra ekosystem kommer fungera allt sämre om förlusten av arter accelererar. Men är dessa slutsatser motiverade? Kan vi förutse ekosystemens funktion baserat på hur hög biodiversitet de har? **Kapitel III** ger inget bestämt svar på den frågan, men höjer ett varningens finger. En av de funktioner som mättes i **Kapitel II** var kvävefixering - omvandlingen av atmosfäriskt kväve till kemiska former som är tillgängliga för andra organismer. Denna process är väl förstådd på molekylär nivå och vi känner till de gener som kodar för de ingående proteinerna. I en tidigare studie har det visat sig att antalet kopior av dessa gener korrelerar väl med kvävefixeringen. För att testa hur allmänt detta resultat är, kvantifierade vi generna i de prover som vi samlade in i **Kapitel II**. Vi försökte sen förutsäga kvävefixeringen baserat på antalet genkopior och förhållandet som hittades i den tidigare studien. Något vi inte lyckades med. I ett andra steg testade vi därför om andra faktorer kunde förklara variationen i kvävefixering. Men varken mångfalden av bakterier eller diversiteten bland endast de bakterier som är involverade i kvävefixeringen korrelerade väl med kvävefixeringen. Medan denna studie bara är ett specialfall, pekar det på hur svårt det är att ta en modell från en studie för att prediktera hur bakteriesamhällen från andra studier fungerar. För att kunna dra generella slutsatser är det viktigt att vi kan validera modeller med oberoende data.

I **Kapitel IV** undersöker jag från ett kritiskt perspektiv ett annat populärt antagande inom området biodiversitet och ekosystemens funktion. Medan

det ofta är tillräckligt med ett fåtal arter för att upprätthålla en funktion, behövs det fler arter för att upprätthålla flera funktioner. Betydelsen av biologisk mångfald föreslås öka med antalet funktioner som vi studerar. Argumentationen är intuitiv. Men, också fel. Trots att det otvivelaktigt är sant att olika arter är bra för olika funktioner, beror nivån på funktionen på arternas abundans. Ta ett enkelt exempel med två arter och två funktioner, där varje art är viktig för var sin funktion. En monokultur av respektive art betyder hög nivå för en funktion, men låg nivå för den andra funktionen. När de två arterna blandas, hamnar båda funktionerna på ett medelvärde av vad de har i monokultur. Argumentet att värdet av biologisk mångfald blir viktigare med antalet funktioner håller inte. Att studera flera ekosystemfunktioner kan vara viktigt i många sammanhang, men det garanterar inte vikten av biodiversitet, och den intuitiva idén behöver därför revideras.

Vad är huvudbudskapet från mitt arbete?

Argumentet att mångfald måste skyddas, eftersom det är avgörande för hur naturen fungerar, är inte generellt och allmänt. Det beror på vilka ekosystem och omvärldsförhållanden vi pratar om. Biologisk mångfald i sig är en inkonsekvent förutsägare för ekosystemens funktionalitet. Detta har dock ingen bäring på vikten av att bevara arter och livsmiljöer. Att skydda ekosystem och de organismer som vi delar vår planet med är en moralisk skyldighet. Från ett mänskligt perspektiv har mitt forskningsområde bidragit till att visa komplexiteten i relationen mellan natur och människa, och hur beroende vi är av väl fungerande ekosystem för vårt välbefinnande. Vi bör därför alltid tillämpa försiktighetsprincipen och undvika de oåterkalleliga förändringar som exempelvis utrotning av arter innebär.

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List of Papers

This thesis is based on the following papers, referred to in the text by their roman numerals.

Paper I **Roger F**, Bertilsson S, Langenheder S, Osman OA, & Gamfeldt L. (2016) Effects of multiple dimensions of bacterial diversity on functioning, stability and multifunctionality. *Ecology*, 97(10), 2716–2728.

Paper II Alsterberg C, **Roger F**, Sundbäck K, Juhanson J, Hulth S, Hallin S, & Gamfeldt L. (2017) Habitat diversity and ecosystem multifunctionality — The importance of direct and indirect effects. *Science Advances*, 3(2), e1601475.

Paper III[1] **Roger F**, Alsterberg C, Wittorf L, Sundbäck K, Hulth S, Hallin S, & Gamfeldt L. (2017) Can we predict ecosystem functioning using tightly linked functional gene diversity? *PeerJ Preprints* 5:e2958v1

Paper IV[2] Gamfeldt L[*], **Roger F**[*]. (2017) Revisiting the biodiversity-ecosystem multifunctionality relationship. *Nature Ecology & Evolution*
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Related publications not included in this thesis:

Roger F, Godhe A, & Gamfeldt L. (2012) Genetic diversity and ecosystem functioning in the face of multiple stressors. *PloS One*, 7(9), e45007.

[1]The paper is published as a Preprint. It has not yet been peer reviewed for formal publication.

[2]This version of the paper is accepted for publication in Nature Ecology & Evolution, but has not yet been proofed and published.

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Section 1

Background

1.1 Introduction

Biodiversity and ecosystem functioning is about the importance of biodiversity. It was born out of the realization that mankind is threatening the amazing diversity of life that evolution has created without us having a scientific understanding of what losing it means. Newbold et al., (2015), estimate that changes in land use already has led to a decline in local species richness of 8% globally and 40% in the worst affected habitats.

In the first book written about biodiversity and ecosystem functioning, Paul Ehrlich titled his foreword *"Biodiversity and Ecosystem Function: Need we know more?"* and answered his own question in two ways: with a clear *"no"*, because we did not need to know more to start protecting biodiversity and with a clear *"yes"* because on the science side of things, there was a very limited understanding of the importance of biodiversity for ecosystem functioning (Schulze and Mooney, 1993). Asking this question can be referred to as "flipping the axes" because instead of asking the traditional ecological question of what governs biodiversity, it asks what consequences do changes in diversity have (Loreau et al., 2002). If an ecosystem loses species, what happens?

This question spurred a surge in theoretical and experimental investigations that totaled more than 900 peer-reviewed publications in 2006 (Solan et al., 2009). The same search, which accurately reproduced the search in Solan et al., yields > 2000 articles if the search period is extended to 2011 (the start of

this PhD thesis) and nearly 4000 articles today (April 2017). In the following, I attempt to give an brief overview of the main findings, the controversies and the recent developments. The summary will necessarily be incomplete. Therefore I choose to spend more space discussing some points that I think deserve increasing attention and where I hope I can contribute to the discussion (Section 3 and 4).

1.2 What diversity and which functioning?

Both terms composing the name of the research field, "biodiversity" and "ecosystem functioning", are often rather loosely defined. "Biodiversity" or "Biological diversity" is defined by the Convention on Biological Diversity as

> *"...the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems."*

For the most part, the literature has focused on inter-specific diversity, mostly measured as species richness - although species number *per se* is often taken to be a stand-in for functional or phenotypic differences (Duffy, 2002; Loreau, 2000). One aim of this thesis is to apply other, more rigorous metrics of diversity and to explore their relationship with ecosystem functioning. I therefore provide a detailed overview about how to quantify and how to estimate taxonomic diversity in Section 3.

But what is the functioning of an ecosystem? On a fundamental level, the role or function of an ecosystem is to sustain the maximum amount of living material per unit time. An ecosystem that sustains more living biomass per unit time for a given set of abiotic (non-living) resources (and under given abiotic conditions) has a higher functioning. Partly in line with this reasoning, biomass has frequently been measured as an ecosystem function—as has nutrient depletion. Yet the range of variables measured as ecosystem function goes above and beyond that. Citing Christensen et al., (1996), Hooper et al., (2005) define ecosystem function as a

> *"variety of phenomena, including ecosystem properties, ecosystem goods, and ecosystem services* [where] *ecosystem properties include both sizes of compartments . . . and rates of processes . . . . Ecosystem goods are those ecosystem properties that have direct market value.. . .* [and] *Ecosystem services are those properties of ecosystems that either directly or indirectly benefit human endeavors . . . "*

Other definitions make the distinction between ecosystem functions (*sensu* ecosystem properties, i.e. standing stock, rates and fluxes) and ecosystem services (properties that benefit humans). In this light, Cardinale et al., (2012) define ecosystem functions as *"ecological processes that control the fluxes of energy, nutrients and organic matter through an environment"* and ecosystem services as *"the suite of benefits that ecosystems provide to humanity"*. It is often implied that ecosystem functions should be value free. Yet, for many variables that are not direct proxies of biomass production, an implicit valuing is mostly inevitable. How else should we decide whether low or high values of variables such as earthworm biomass or carbon storage represent low or high functioning for an ecosystem? In practice, the term ecosystem function has been used very broadly and what constitutes an ecosystem function has for the most part been in the eye of the beholder.

The other aspect that is usually comprised into the concept of ecosystem functioning is ecosystem stability. In ecology, stability has many different meanings (Pimm, 1984). In ecosystem function research, the focus has been on stability *sensu* the temporal variability of stocks or process rates (e.g. biomass or respiration) and the resistance or resilience of these stocks and processes to perturbations.

1.3 How can diversity affect ecosystem functioning?

There is a range of ways in which changes in biodiversity are predicted to affect ecosystem functioning. The main effects can be broadly characterized along two axes - whether the effect is (largely) biological or (largely) statistical and whether the effect acts on the magnitude of ecosystem functioning

or on the temporal or spatial stability (Fig. 1.1). The most important biological effect is niche complementarity, which is the basis of the **complementarity effect** (Loreau and Hector, 2001; Tilman et al., 1997a). It lies at the heart of the biodiversity-ecosystem functioning hypothesis: species have different requirements, both in terms of resources and in terms of the physiochemical conditions in which they thrive. A plant species with taproots might access water and nutrient reserves that are inaccessible to a species with fibrous roots, while a species with fibrous roots is more efficient in using shallow resources. A shade-tolerant fern can thrive under the dense canopy of shade-intolerant trees. In wave-exposed intertidal rocky shores, a zonation of macroalgae is the product of the ability of different species to occupy different niches in a niche space defined by gradients in wave exposure, light, risk of desiccation, and susceptibility to predation. In none of the provided examples can a single species occupy the full niche space. Therefore, a diverse set of species can utilize a greater niche space and more of the available resources than can any single species. The complementarity effect refers mostly to the local spatial niche space and to its ability to increase the magnitude of local ecosystem functioning. Besides niche-partitioning, it also includes positive interactions among species.

The **insurance hypothesis** Yachi and Loreau, 1999 relies on the same mechanism of niche partitioning but with respect to temporal niche differentiation and its effect on the temporal stability of ecosystem functioning. Under fluctuating environmental conditions, different species can thrive—and uphold functioning—at different times. In this scenario, the abundance of single species fluctuate, driven by the fluctuating environmental conditions, but overall community biomass is stabilized. This is true in general: the mean of different fluctuating entities has a smaller temporal variance than each individual entity—as long as the fluctuations are not synchronous. This purely mathematical effect is agnostic to the cause of fluctuations and is called the **portfolio effect** (Doak et al., 1998)—in analogy to the investment strategy to spread the assets to stabilize the return. As such, the insurance hypothesis can be seen as a special case of the portfolio effect where the focus lies on the cause of asynchrony, i.e. adaptation to different environmental conditions. The spatial variant of the insurance effect is proposed to operate in metacommunities where adapted species can disperse between communities and

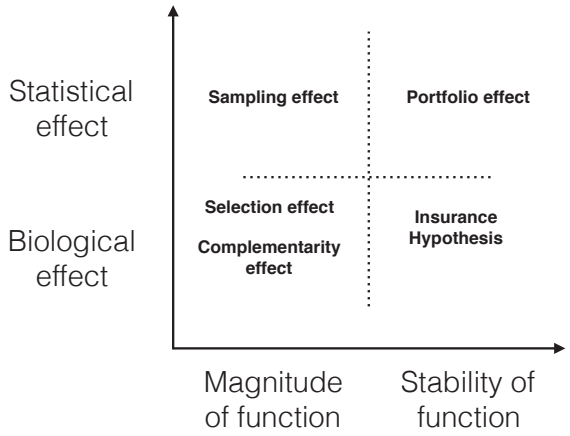

FIGURE 1.1: Biodiversity effects can be broadly characterized as (largely) biological or (largely) statistical.

thereby stabilize ecosystem functioning (Loreau et al., 2003).

Another statistical effect is the **sampling effect** (Huston, 1997; Tilman et al., 1997a). It describes the fact that a more species-rich community has a higher probability of including a species with extreme trait values, which dominates process rates. It has been described as a statistical artefact or *"hidden treatment"* (Huston, 1997) of biodiversity experiments—especially as natural community assembly is not random. Yet, the sampling of a species with a dominant trait alone is not enough to generate a diversity effect—the species must also be "selected for". A positive correlation between competitive advantage and positive trait values is assumed in the sampling effect, but this is not necessarily true. Therefore, Loreau, (2000), suggested the term **selection effect** (that can be both positive and negative). The selection effect describes the general case where there is a relation between the trait value of species and their competitive success in polycultures.

Note that the two-axis categorization that I present is not absolute. The definitions of each effect vary to some extent. As such, the insurance hypothesis can also act on the magnitude of the temporal mean—if it is coupled with a positive selection effect. And the selection effect, complementarity effect and insurance hypothesis all rely on the sampling effect to some extent.

1.4 How does diversity affect ecosystem functioning?

1.4.1 Evidence from experimental data

A range of reviews have summarized the findings of biodiversity and ecosystem functioning research. Here I focus on the most recent set of quantitative reviews by Cardinale et al., (2011), Cardinale et al., (2012), Griffin et al., (2013), and Gamfeldt et al., (2015). All but one focus on the effects of richness (mainly species richness and to a lesser degree genotype or functional group richness) with the exception being Griffin et al., which also investigated the effect of taxonomic distinctiveness.

Cardinale et al., (2011), focused on the functional role of primary producer diversity in both terrestrial and aquatic systems. The authors present the results in the light of species loss, but as the evidence is overwhelmingly based on assembly experiments, and not removal experiments, I choose to present the results as a function of changes in species richness more generally. The majority of experiments show that the average standing stock biomass of producer communities increases with richness, as does the average nutrient assimilation efficiency. There are some studies suggesting that actual rates of primary production increases with species richness but the data are scarce. The authors find strong evidence that both selection and complementarity effects are important. This is based on studies that used the framework of Loreau et al., (2002), to partition net diversity effects into selection and complementarity effects—which compares monoculture yields to the yields achieved in mixtures. However, the authors note that "complementarity" as measured by this framework does not necessarily result from niche partitioning. The studies summarized by Cardinale et al., (2011), also show that in the majority of cases, mixtures were outperformed by the best monocultures. The most common shape of the relationship is a positive and saturating curve.

The review by Cardinale et al., (2012), expanded the focus to all published biodiversity and ecosystem functioning experiments (including, but not exclusively focusing on, primary producers). The authors come to similar conclusions as Cardinale et al., (2011). They add that *"there is mounting evidence*

that biodiversity increases the stability of ecosystem functions", which is corroborated by Isbell et al., (2015), who report that stability and resistance (but not resilience), in face of climate extremes, are higher at high diversity in grassland experiments. Cardinale et al., (2012) also suggest that diversity loss across trophic levels might have stronger effects than diversity loss within trophic levels.

Griffin et al., (2013), focus on the effect of predator richness. They find that in the majority of cases, predator richness enhances prey consumption over the average single predator community, but not over the best-performing single predator community. The strength of the positive effect increased with taxonomic distinctiveness of the predator assemblage.

Gamfeldt et al., (2015), focus on biodiversity and ecosystem functioning studies conducted in marine systems. Here, for all three types of studied ecosystem functions (production, consumption and biogeochemical fluxes), the most diverse polycultures outperform the average monocultures but are on par with—or outperformed by—the highest-functioning monocultures. The relationship between species richness and average ecosystem functioning is linear for production and saturating for consumption.

The authors of all four reviews speculate that stronger diversity effects might be observed at larger temporal and or spatial scales—as the scope for niche complementarity increases with more heterogeneity. Cardinale et al., (2011) and Griffin et al., (2013), tested this hypothesis with the available data and found some support for it. Meyer et al., (2016), studied the change of local diversity effects through time and found that for 14 of 50 investigated variables (28%), the diversity effect strengthened, mainly because of lower performance of the monocultures over time.

The magnitude of the effect of potential species richness loss on productivity was assessed by Hooper et al., (2012). The authors compiled data from studies that investigated the richness - productivity relationship and plotted the observed effect size at each richness level against what percentage the given richness level represented compared to the highest richness level. The

authors discussed this as percentage species loss although strictly speaking the underlying experiments were based on species assembly, not removal. With that caveat in mind the authors conclude that the effect of diversity loss depends on the extent of the loss, which could rival the effect of stressors such as ultraviolet radiation or warming for intermediate losses (40%) and the effect of severe stressors such as drought for the highest losses (80%). Similar results were found in an analysis of a long-term grassland experiment (Tilman et al., 2012) where the difference in production between sites with 1 and 16 species was larger than for any other stressor (water, drought, CO_2 and herbivore exclusion).

Overall, the experimental evidence is remarkably consistent. The vast majority of studies finds that the most diverse polycultures outperform the average—but not the best—monoculture. The relationship is saturating, with a rather steep increase with the addition of the first few species and smaller increases thereafter.

1.4.2 Evidence from observational data

While the general conclusion is widely acknowledged, the relevance for natural ecosystems has been criticized. The usefulness of the experimental approach has been called into question again recently (Wardle, 2016): Wardle argues that (i) species assemblages are not random subsets of a regional species pool, and species are not lost in a random fashion in real ecosystems, (ii) while there is no doubt that species are lost globally, there is less evidence showing that local species richness is declining, and (iii) that the context dependency of the relationships is not sufficiently acknowledged, limiting the ability to predict concrete outcomes from expected species loss (but see Eisenhauer et al., (2016), for a response).

Partly in line with this criticism, results from natural experiments have been variable. Natural gradients of species richness on islands in northern Sweden show no consistent relationship between species richness and productivity (Wardle et al., 1997). Removal experiments in the same study system reveal that species richness and functional group richness are important

but highly context-dependent (Wardle and Zackrisson, 2005). Using structural equation modelling to disentangle the effects and interrelationships of abiotic factors, local species richness, standing biomass, and disturbances in grasslands across the globe, Grace et al., (2007), find no relationship between species richness and biomass. In contrast, Mora et al., (2011), report a strong relationship between coral reef fish functional richness and standing stock fish biomass—a finding corroborated by Duffy et al., (2016) who report that fish species richness and functional diversity were the strongest predictors of fish biomass in tropical reef ecosystems (along with temperature). Positive diversity-ecosystem functioning relationships have also been found for drylands (Maestre et al., 2012) and forests (Gamfeldt et al., 2013; Paquette and Messier, 2011; Vila et al., 2007). However, many relationships are relatively weak (e.g. Maestre et al., 2012), and not universal (Burley et al., 2016).

1.4.3 Evidence from different levels of diversity

The overwhelming majority of studies has considered the effects of changes in species richness on ecosystem functioning. Yet, the concept of biodiversity also includes the variation at smaller and larger scales of organisation, such as genetic and habitat diversity. Some evidence for the potential benefits of genetic diversity comes from seagrass ecosystems. Experimental manipulations of genotype diversity suggests positive effects on a variety of variables, including primary production, resistance to grazing by geese, and resilience to heat-waves (reviewed in Duffy et al., 2014). Studies in other systems show positive effects of genetic diversity on pest resistance in rice (Zhu et al., 2000), increased productivity (Bell, 1991) and positive complementarity in algal cultures (Roger et al., 2012) as well as a range of other ecosystem functions (Hughes et al., 2008). The role of habitat diversity *per se* has not been investigated to date in the framework of biodiversity and ecosystem functioning research (but see **Chapter II** in this thesis).

1.4.4 Considering multiple functions

In the last decade, the focus of biodiversity and ecosystem functioning research has largely shifted from the question of how biodiversity affects single functions to how diversity can affect multiple functions simultaneously—so

called multifunctionality. Any given ecosystem performs more than one function or provides more than one service. To get a full picture of how ecosystem functioning is affected by any factor, multiple functions need to be considered. It follows, that if we want to quantify the importance of biodiversity for ecosystem functioning we should likewise consider its importance for the simultaneous provision of multiple ecosystem functions. The common expectation is, that—as species perform different functions and/or the same functions at different levels—biodiversity should be more important for overall functioning if more functions are considered. Therefore multifunctionality is frequently suggested as solution to the conundrum that single functions are frequently maximized by single species or saturate at low richness levels (Byrnes et al., 2014; Duffy, 2009; Gamfeldt et al., 2008; He et al., 2009; Hector and Bagchi, 2007; Isbell et al., 2011; Lefcheck et al., 2015; Mouillot et al., 2011; van der Plas et al., 2016; Zavaleta et al., 2010). Utilizing a range of different methods, many influential papers published in the last decade came to this conclusion. A recent meta-analysis found general positive effects of biodiversity on multifunctionality and reported a stronger diversity - multifunctionality relationship when more functions were considered (Lefcheck et al., 2015).

Section 2

This Thesis

Science is not a one-person show and this thesis is no exception. None of the chapters described below would have been possible without my co-authors. While I lead the collaborations in Chapter I and Chapter II, co-lead Chapter IV and contributed significantly to Chapter III, I settle for "we" as a personal pronoun. Note that "we" denotes a variable group of co-authors, depending on the chapter.

2.1 Chapter I

In **Chapter I** we assess the importance of diversity in mega-diverse microbial systems. There is ample evidence that changes in diversity affect ecosystem functioning but the evidence is mostly based on experiments manipulating only a few species. Among the marine experiments reviewed in Gamfeldt et al., (2015), the median number of species in the highest richness level is three. Most natural ecosystems are orders of magnitude more diverse but we know little about the consequences of diversity loss in highly diverse communities. Such diversity is difficult to manipulate. Bacterial communities, however, are exceptions as diversity gradients can be created through sequential dilution—so called dilution-to-extinction. The method is illustrated in Fig.2.1. Bacterial communities are characterized by a steep rank-abundance curve, and in a dilution series, rare species are lost sequentially—which creates a diversity gradient.

We used pelagic bacterial communities that were collected from four lakes. The communities were diluted to create a diversity gradient and incubated outdoors in large water tanks (mimicking the temperature variations in the

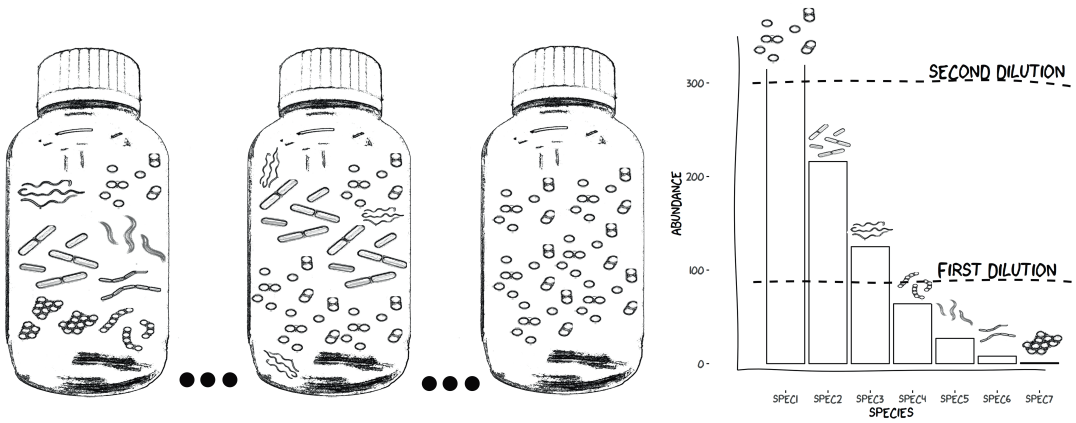

FIGURE 2.1: An illustration of the dilution-to-extinction approach to create a gradient in diversity. For species assemblages characterized by a steep rank-abundance curve, rare species are lost sequentially through a dilution series.

surface water of lakes). The experiment was run for six weeks. Both the long duration of the experiment (relative to the short generation times of bacteria) and natural temperature fluctuations are theoretically predicted to increase the chance for diversity effects. A setup of the experiment is shown in Fig.2.2.

While the approach of dilution-to-extinction is not new, until relatively recently it was not possible to accurately quantify the realized diversity gradients. Here, as in **Chapter II** and **Chapter III**, we leverage the development of next-generation sequencing techniques to assess diversity and community composition. This also allowed us to investigate more relevant metrics of diversity, taking into account the phylogenetic relationship among bacteria and their relative abundances. In addition, we measured functional diversity with a community profiling assay (Biolog EcoPlates).

We related three metrics of diversity (effective number of species, phylogenetic diversity, and functional diversity) to three response variables: (i) bacterial abundance, (ii) stability of bacterial abundance, and (iii) water nitrogen concentration. We also analysed multifunctionality (the three response variables considered jointly). The experiment showed little evidence that diversity matters for ecosystem functioning or multifunctionality. This was true

FIGURE 2.2: Areal photo of the experimental set-up in Chapter I.

regardless of diversity metric. Our results were corroborated by a literature review of 21 peer-reviewed studies that also used dilution-to-extinction to manipulate bacterial diversity: only 25% of the experiments in these studies found positive relationships. Combined, the results suggest that bacterial communities are able to uphold a range of ecosystem functions even at extensive reductions in diversity.

2.2 Chapter II

In **Chapter II** we experimentally investigated the potential effects of habitat diversity on ecosystem functioning. The relationship between biodiversity and ecosystem functioning has been shown to be stronger in the context of higher habitat heterogeneity, e.g. with higher spatial heterogeneity of limiting resources or higher structural diversity of the substrate (Angelini et al., 2015; Griffin et al., 2009b; Tylianakis et al., 2008). The supposed mechanism is that heterogeneity increases total niche space and thereby also increases the potential for species complementarity. We also know that habitats are coupled via the migration of individuals or the passive physical transport of

materials—e.g. between lakes and the riparian ecosystem surrounding them (Schindler and Scheuerell, 2002), or between the benthos and the pelagic in the ocean (Darnis et al., 2012).

The effects of habitat diversity *per se* on ecosystem functioning, i.e. the effect of diversity of habitat types within a landscape, are largely unexplored. We hypothesised that habitats can facilitate each other, just as species can, and that landscape-wide ecosystem functioning can be promoted by habitat complementarity. An example is the interplay of mangrove, seagrass and coral reef habitats in tropical coastal waters. Mangrove forests capture sediments and reduce water turbidity, which is beneficial for both seagrasses and corals. Seagrass meadows further reduce water turbidity and sedimentation, and filter excess nutrients from the water column—which limits the growth of macroalgae. Coral reefs, in turn, provide physical protection against wave exposure and erosion to both seagrasses and mangroves.

Manipulating habitats experimentally is often not feasible. Therefore natural microbial systems represent a unique opportunity. For **chapter II**, we worked with shallow marine sediments as model system. In this system, habitat-defining characteristics for microorganisms vary over small spatial scales and different types of sediment can represent different environments. The same is true for the presence of dominant keystone taxa, like the seagrass *Ruppia maritima* and mat-forming cyanobacteria. Since many important functions in shallow marine bays are driven by microorganisms (e.g. nutrient cycling), process rates, too, can vary on small scales. Importantly, marine sediment habitats are connected through the overlying water and thus exchange nutrients and material.

We assembled sediment cores from four different habitat types (sandy sediment, silty sediment, sediment with *Ruppia maritima* and sediment with cyanobacterial mats) into artificial landscapes with varying habitat richness (1 - 4 habitats) Fig.2.3. We measured four biogeochemical processes: gross primary production, nitrogen fixation, denitrification, and uptake of dissolved inorganic nitrogen. Bacteria and archaea are partly or solely responsible for each of those processes. As different species are likely to be present in different habitats, microbial diversity is expected to be higher in landscapes containing

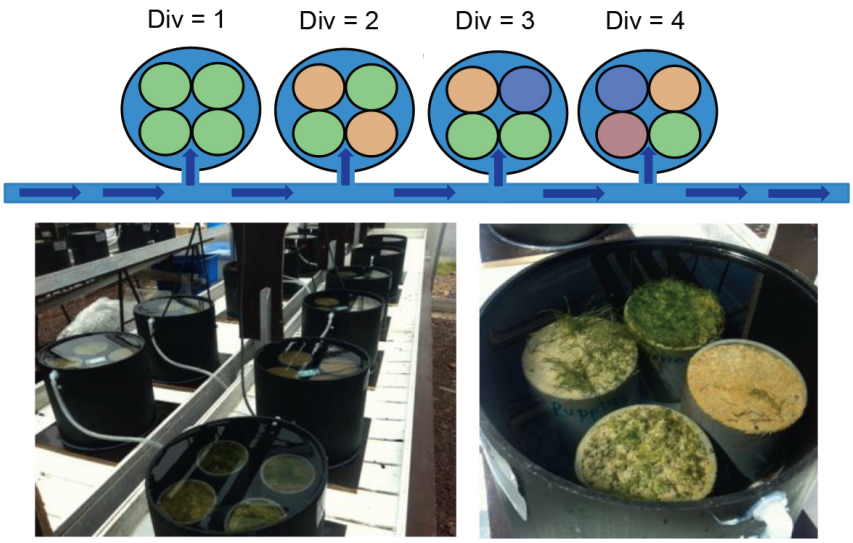

FIGURE 2.3: Experimental set-up of the experiment in Chapter II.

more habitat types. To disentangle the potential effects of microbial diversity from the effects of habitat diversity we estimated bacterial and archeal diversity via amplicon sequencing and calculated phylogenetic diversity (*sensu* Chao et al., 2010). For habitat diversity, we did not assume that each habitat was equally different from each other, nor that landscapes containing four cores with the same habitat type had no intra-habitat variations. Instead, we characterised each habitat by characteristics such as porosity, carbon and nitrogen content, and microalgal pigments. Based on these characteristics, we calculated a distance-based metric of habitat diversity. Using structural equation models to statistically disentangle the effects of habitat diversity and microbial diversity we show that landscapes constituted by a diversity of habitats have higher levels of multifunctionality than those with low habitat diversity. This effect is both direct, through positive interactions among habitats, and indirect, via increased species diversity—depending on season.

Notably, the direct effect of habitat diversity must be due to positive interactions. A selection effect can be excluded, because for a selection effect to be present, the relative proportion of habitats would have to change over time

(which was not possible in our setting). Likewise, complementarity caused by niche partitioning can be ruled out as explanation for a positive diversity effect: species partition niches within habitats but there is no habitat for habitats. Therefore increased multifunctionality in the four-habitat treatment compared to the single habitat-treatment can only be caused by positive interactions among habitats. Yet, here too, the high diversity treatment only outperformed the average single habitat but not the highest performing single habitat. Thus we observe the same pattern in this first example of habitat diversity as has been observed in the majority of studies focusing on species diversity.

2.3 Chapter III

In **Chapter III** we ask the question whether detailed knowledge about the microbial community allows us to make predictions for process rates.

The finding that biodiversity increases ecosystem functioning above the average of single species is general. This has spurred verbal predictions that future loss of diversity will have adverse consequences for ecosystem services and human well being (Cardinale et al., 2012). The few quantitative predictions that have been made (Cardinale et al., 2011; Gamfeldt et al., 2015; Hooper et al., 2012; O'Connor et al., 2017) have not been validated on independent data, and we cannot know how accurate they are. Furthermore, due to the limitations of the underlying data, the predictions are—strictly speaking—predicting the outcome of typical diversity experiments rather than of biodiversity loss in real ecosystems.

Houlahan et al., (2017) argue that in absence of verified prediction we cannot demonstrate understanding. Following that logic, we ask whether detailed knowledge about the microbial community allows us to inform our expectation about observed process rates. In **Chapter II**, nitrogen fixation varied considerably among samples. While habitat diversity provided explanatory power, the residual variation was large. Nitrogen fixation,the biological transformation of atmospheric nitrogen gas into bioavailable ammonium, is a crucial ecosystem function—and this process is exclusively performed by

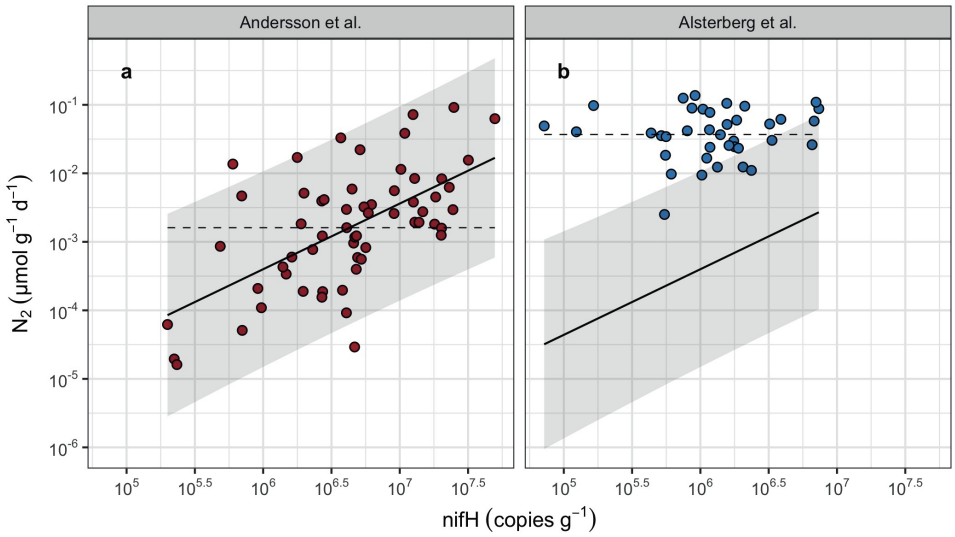

FIGURE 2.4: Predicting nitrogen fixation by the abundance of the *nifH* gene in marine shallow water sediments. **a** Data from the previous study Andersson et al., and **b** nitrogen fixation data from **Chapter II**.

free-living and symbiotic bacteria and archaea (diazotrophs). Moreover, the genes encoding the proteins that perform nitrogen fixation are known and shared by all diazotrophs. Thus, to study diazotrophs, it is established praxis to study the *nifH* gene, which encodes the enzyme dinitrogenase reductase involved in the process. Given that, **Chapter III** has two objectives: In a paper by Andersson et al., (2014) it was observed that nitrogen fixation in sediments from shallow marine bays along the Swedish west coast was linked to the abundance of the *nifH* gene. The first objective was to validate that relationship on the independent data we collected in **Chapter II**. We quantified the *nifH* genes from DNA samples collected in **Chapter II** and predicted expected nitrogen fixation rates based on the relationship observed for the data from Andersson et al. We found that, while the statistical relationship found in Andersson et al. was reasonably strong (R2 = 0.37, p = 2.9×10^{-7}), it had no predictive power on our independent data (Fig.2.4).

The second objective was to sequence the denitrifier community and test whether the diversity of denitrifiers (expressed as effective number of *nifH*

OTUs), or the abundance of certain phylotypes, correlated with nitrogen fixation rates. As data on the general bacterial community (based on 16S rRNA sequencing) were available from the previous study, we also tested for a correlation with the general bacterial diversity. None of the tested community metrics correlated with the nitrogen fixation rates.

Our study provides a cautionary tale for the generality of correlative findings. It also showed, as **Chapter I**, that bacterial diversity explained little of the variance in observed process rates. We conclude that while this study provides a special case, the point is general: unless we can show that prior knowledge of community metrics informs our expectation of ecosystem functioning, the link remains elusive and speculative.

2.4 Chapter IV

A recent and prominent claim for the value of biodiversity is its role in simultaneously sustaining multiple ecosystem functions. The general idea is appealing and intuitive: since all species are to some extent unique, they will be important for different functions. Thus, as more dimensions of functioning are considered, the value of a high diversity of species becomes more apparent.

The concept of biodiversity and multifunctionality has become quite popular in ecology, conservation and management. Since the publication of what can be referred to as the biodiversity-multifunctionality "foundation" paper in 2007 (Hector and Bagchi, 2007), there has been an exponential increase in both the number of papers and citations. A search on Web of Science using the search query "biodiversity AND multifunctionality" reveals 40 papers published in 2015, and an accumulated number of citations of more than 1100 since 2007. The vast majority of these studies, if not all, rest on the same assumption: biodiversity can causally beget multifunctionality.

In **Chapter IV** we argue that we should rethink the idea that biodiversity positively impacts multifunctionality beyond its effects on single functions.

With simple models we make it clear that, contrary to common belief, increasing the number of functions considered cannot by itself change the nature of the biodiversity-functioning relationship. Because of trade-offs, some ecosystem functions will be provided at high levels at the expense of other functions. It is a zero-sum game. Biodiversity can only affect the level of multifunctionality by impacting individual functions.

We also caution against the use of a popular multifunctionality metric—the multiple threshold approach—as we show that it behaves inconsistently.

Section 3

Measuring and Estimating Diversity

Accurately measuring diversity in natural communities was one focus of this thesis and has been central to **Chapters I, II** and **III**. This includes moving away from using species richness as a metric of species diversity (towards phylogenetic diversity and to some extent functional diversity) and incorporating abundance information by expressing diversity in units of effective numbers. It also includes reflecting upon the challenges of estimating diversity in natural ecosystems in general—and in bacterial ecosystems, from sequencing data—in particular. I therefore discuss the topic in some depth in this Section.

In **Chapter IV** I caution against the use of the multithreshold approach but do not offer an alternative (which would have gone beyond the scope of the chapter). Therefore I take the opportunity to outline some ideas of what a better metric of multifunctionality should and could be in Section 4. Drawing the parallel between measuring the diversity of species and the diversity of functions, I suggest that it might be possible to develop a new metric of multifunctionality based on some of the same methods that I recommend for measuring species diversity. I also summerize some thoughts regarding the inherent limitations of any multifunctionality metric.

3.1 Three dimensions of diversity

3.1.1 Species richness

The by far most common metric of diversity has been, and still is, **species richness**, i.e. number of species in an assemblage. This is somewhat surprising as evidently, taken at face value, species richness cannot be a universal predictor for ecosystem function. As pointed out by Jan Bengtsson in an early critique (Bengtsson, 1998) *"the use of species number as an indicator of an ecosystem's diversity suggests that all species are potentially equal with respect to function"*. Bengtsson goes on to ask what the equivalence of one earthworm species would be in units of species of mites or fungi. The answer is partly that no study on biodiversity and ecosystem functioning considers the whole species pool. More typically, experiments assemble communities from a regional species pool and test different combinations of these species in assemblages of different richness. Yet, even in such a scenario it is not clear what ecological mechanism would make the number of species a good predictor (besides maybe for the sampling effect). Hence, the most sensible reason to use species richness as measure of diversity is as surrogate or proxy for other dimensions of diversity, most notably functional richness.

3.1.2 Functional diversity

If niche partitioning is assumed to underly a positive diversity effect on ecosystem functioning, species have to be maximally different (within a given niche space) to make use of the greatest amount of available niche space (Díaz and Cabido, 2001; Tilman et al., 1997b). Yet, species richness is not necessarily linearly related to the occupation of niche space, unless species are drawn from a species pool with random trait values or from a species pool in which trait values are distributed uniformly over the niche space (Díaz and Cabido, 2001). In all other cases, it should be preferable to measure the amount of covered niche space directly, which is what functional diversity attempts to do.

The importance of functional diversity was recognized from the beginning (Schulze and Mooney, 1993) and was part of the first experiments (Tilman et

al., 1997b). Yet, how to define and how to measure functional diversity correctly is debated. The most common way to quantify functional diversity has been to categorize species into functional groups based on their traits, and express functional diversity as number of functional groups (i.e. functional group richness). This is problematic for several reasons (Petchey and Gaston, 2002b, 2006; Petchey et al., 2004). For one, most traits are continuous and a categorization into discrete classes will be inevitably arbitrary. This also implies that depending on how differences are defined, any given species assemblage can be either lumped together in a single functional group or subdivided so that each single species forms its own group. Second, information about within-group variation is lost and only the information about between-group differences is kept. Third, which relates to the first point, just as for species, functional groups are then regarded as equivalent and equidistant, which is likely an unjustifiable assumption. These problems can be circumvented by using metrics of functional diversity that capture the continuous and multidimensional nature of the trait values underlying functional diversity, such as FD (Petchey and Gaston, 2002b) or $FRic$ (Villéger et al., 2008). FD, named in analogy to the metric of phylogenetic diversity PD (Faith, 1992) (see below) measures functional diversity as the branch length of a cladogram relating all species in a community, hierarchically clustered based on their trait values. $FRic$, or functional richness, quantifies functional diversity as convex hull volume in multidimensional trait space. Yet, neither of the two alternatives solve a different, more fundamental problem with functional diversity, i.e. what traits to measure. Petchey and Gaston, (2006) answer the question as *"the correct number of traits is the number that are functionally important"*. The problem with that answer is multifaceted: 1) as pointed out by (Bengtsson, 1998), there is a certain degree of circularity in this reasoning. If we relate ecosystem functioning to the diversity of traits that we choose *a priori* based on their importance for the function we measure, we are not longer talking about functional diversity as an independent variable. This might be especially problematic if the selection is not based on independent ecological information, but as the set of traits that maximize the explained variance. This highlights the second problem: what traits to include and based on what criteria? The possibility of a subjective choice leaves researchers a tempting amount of *"researcher degrees of freedom"* (Simmons et al., 2011) where any set of traits that yields results in agreement with the original hypothesis can be

justified by motivated reasoning. Third, how can we assume that we know all relevant traits important for a given function, and even if we were to know them, how could we be sure that we can measure them all? Fourth, species traits are not necessarily constant over time and space and can be separated into effect traits and response traits (Lavorel and Garnier, 2002).

3.1.3 Phylogenetic diversity

Some of these problems have lead to the emergence of another way to measure functional differences between species without relying on measuring (a subset of sometimes deemed arbitrary) functional traits: Phylogenetic diversity. Phylogenetic diversity was first suggested as metric to help guide conservation priorities (Faith, 1992). Faith motivates its value in the ability to guide conservation decisions so that limited resources can be focused *"such that the subset of taxa that is protected has maximum underlying feature diversity."* (Faith, 1992). *"Feature diversity"* here is synonymous to species' traits and the underlying assumption is that how similar species (or even individuals) are in regard to their traits can be predicted based on their phylogenetic relatedness. The same reasoning led (Cadotte et al., 2008) to suggest that phylogenetic diversity should bear information about the trait space used by species and hence be a good predictor of ecosystem functioning. However, phylogenetic diversity, too, has limitations that need to be considered and which are summarized by Srivastava et al., (2012) and Mouquet et al., (2012). I discuss the two main problems below.

First, for phylogenetic diversity to be a good proxy of functional diversity, the relevant traits for the ecosystem function under consideration must have a strong phylogenetic signal. This might best be illustrated with an example: imagine that we are interested in the important ecosystem function of grazing in coral reefs. We know that grazers are somewhat specialized and hypothesize, based on that, that a diverse assemblage of grazers grazes more efficiently than any given species alone. If—for one reason or another—it is difficult to measure what the different grazers actually consume, we might assume instead that we can approximate "similarity in food preference" by the phylogenetic relatedness between any pair of grazers. In other words, we assume that two closely related grazer are more likely to eat

the same algae than two distantly related grazers. If we are right, then we say that the trait of "food preference" has a phylogenetic signal and phylogenetic diversity might be a decent proxy for total occupied "grazing niche space"—which might be predictive of the actual ecosystem function of grazing efficiency. But we might as well be wrong. As noted by Srivastava et al., (2012), closely related species might have evolved by adaptive radiation. For bacteria, it could even be shown that experimental evolution, mimicking adaptive radiation, had the potential to overwrite a previously detected signal of phylogenetic trait conservation (Gravel et al., 2011). But—and this is the second problem—even if the assumption generally holds, taking into account all species in a large phylogeny, the relationship can be blurred locally because of community assembly mechanisms. In our example, imagine that a $phylogenetic\ distance \sim trait\ distance$ relationship is driven by phylogenetically distant clades that have very distinct traits. Now, if only one of the clades is present locally, the relationship might not hold within that clade. Other examples include when the environment selects for very similar species or very dissimilar species. In both cases, the relationship might be less strong than if the full species pool was taken into account. Srivastava et al., (2012), conclude that *"Ultimately, the utility of PD will depend on whether the functional traits of particular importance for ecosystem functioning happen to be the same as those whose phylogenetic signal is preserved at the community scale."*

3.1.4 Which metric predicts ecosystem functioning best?

There has been some debate in the literature about which metric is the best predictor of ecosystem functioning. Metrics of functional diversity have been reported to outperform other metrics in grassland experiments (Petchey et al., 2004), and rock-pools with macroalgae (Griffin et al., 2009a). A meta-analysis of grassland experiments found that phylogenetic diversity was a better predictor of community biomass than species richness (Cadotte et al., 2008, 2009). These findings sparked a vivid debate, as other authors maintained that species richness was in fact the better predictor (Cadotte, 2015; Cardinale et al., 2015; Venail et al., 2015). It is notable, however, that much of the underlying methodological disagreements was spurred by the very high colinearity of PD and richness (R2 of 0.9) and that while the original analysis by Cadotte et al., (2008), found PD to be a better predictor, "better"

referred to 2% more variance explained. But the debate also relied on different lines of evidence: Cadotte, (2013), manipulated PD explicitly (in contrast to the experiments disputed above) and found it to be a significant and good predictor for biomass production. In contrast, in natural and experimental freshwater green-algae assemblages, phylogenetic diversity was unrelated to co-occurrence or competitive outcomes (Alexandrou et al., 2014; Narwani et al., 2013; Naughton et al., 2015).

I think it is fair to say that to date, no consensus has emerged. More importantly however, it is not clear to me if the question should be answered by quantitative arguments. Functional diversity is the only metric that can claim to measure a property that is directly and logically linked to a mechanism underlying ecosystem functioning, i.e. niche complementarity—if the right traits are measured in the right way. As discussed above, these caveats make functional diversity a difficult metric to use in practice. The proponents of phylogenetic diversity do not claim that PD is causally linked to ecosystem functioning (Cadotte et al., 2011), but suggest that it has the potential to be a better proxy for true functional diversity than functional diversity itself. This might be true if certain assumptions are met (see above). Richness on the other hand seems to do neither. Although sometimes proponed as such, it is not an obvious stand-in for functional diversity nor can it be logically and causally linked to ecosystem functioning. It is somewhat unclear to me where the merit lies to demonstrate that in a given set of grassland experiments, richness explains a higher proportion of variance than alternative metrics. While functional diversity is linked to a clearly defined hypothesis (e.g. which traits are important), and phylogenetic diversity has testable assumptions (i.e. relevant traits must have a phylogenetic signal in the local community), richness does or has neither. If we manipulate richness it is often unclear what mechanistic ecological hypothesis we test.

3.2 What *is* diversity?

One aspect that we have ignored in the previous discussion about diversity metrics is the question about relative abundance. All of the metrics

mentioned above, be it species richness, functional richness, functional diversity (*sensu* Petchey and Gaston, 2002a) or phylogenetic diversity (sensu Faith, 1992) are metrics of "richness", not diversity in the broader sense. They weigh all species equally regardless of their abundance. This means that two communities containing ten species each and a total of hundred individuals, always have the same diversity according to those metrics. This is true, even if one community has ten individuals from each species and the other ninety-one from the same species with a single individual from each remaining species. This runs counter to our intuitive understanding. While we might perceive the first community as diverse (picture a forest with ten species, roughly in the same abundance, in the fall, where all species have leafs of different colours), we would be hesitant to qualify the second community as equally diverse or even diverse at all (picture a planted spruce monoculture with nine small individual trees of different species that have settled on the outskirt of the forest). In the words of Hill, (1973):

> *"When we say that the humid tropics are more diverse than the tundra, we mean that there are more species there. More precisely, we mean that the species in the humid tropics have on average lower proportional abundances than those in the tundra."*

From a biodiversity-ecosystem functioning perspective, it also makes little sense to ignore relative abundances. The best guess as to how individual species affect ecosystem functioning is that they do so proportional to their abundance. Therefore ecologist have long used metrics of diversity that incorporate information about relative abundance (although it has not been the default in the field of biodiversity and ecosystem functioning). Two of the most common metrics are Shannon entropy and the Simpson index. The Shannon entropy gives the uncertainty about the species identity of a randomly chosen individual. The Simpson index gives the probability that two randomly chosen individuals from the species assemblage belong not to the same species. Both metrics are abundance-sensitive. The Shannon entropy weighs species by their proportional abundance, and the Simpson index by the square of their proportional abundances. Yet, as pointed out by Jost, (2006), both lack crucial properties that one would intuitively expect from a measure of diversity, the most important being the "doubling property" (or in its generalized form, the "replication principle").

Again, imagine two communities both having ten species in equal abundance but having no species in common. The Shannon entropy (with base 2) is 3.3 for each community, and the Simpson index is 0.9. What do we get if we pool the communities and hence have a new community with 20 different species, all equally abundant? The Shannon entropy of the pooled community is 4.3 and the Simpson index is 0.95. Neither of the two indices is doubled, while objectively the diversity has doubled. In fact, the Simpson index merely increased by 0.05 or, 5.5%. It is therefore very unintuitive to compare two communities of different diversity based on these metrics. Yet, we would say that any two communities having the same Shannon or the same Simpson index are equally diverse. Based on this premise we can derive a more intuitive metric of diversity (Jost, 2006). There is an infinite number of communities with the exact same Shannon or Simpson index that differ in their richness and their relative abundance distributions. Among these communities, there will be exactly one where all species are equally abundant. The number of species in *that* community is called the *effective number of species* (Hill, 1973; Jost, 2006; MacArthur, 1965). In the words of Jost, (2006):

> *"In physics, economics, information theory, and other sciences, the distinction between the entropy of a system and the effective number of elements of a system is fundamental. It is this latter number, not the entropy, that is at the core of the concept of diversity in biology."*

Hill, (1973), and Jost, (2006), have shown that the Shannon entropy and Simpson index are special cases of generalized entropies that differ only in their weighing of the proportional abundance q. In fact, this is true for most of the common "diversity" indices used in biology, including Shannon entropy, all Simpson measures, all Renyi entropies, all HCDT or "Tsallis" entropies *and* species richness (Jost, 2006). All can be expressed as generalized entropies that can be converted to effective number of species of "order" q with the following formula:

$$D_q = \left(\sum_{i=1}^{S} p_i^q \right)^{\frac{1}{1-q}} \tag{3.1}$$

Where p_i is the relative abundance of the i^{th} species and q is the weight given to the species' relative abundances. Species richness, the effective number of species based on Shannon entropy, and the effective number of species based on the Simpson index, and even the Berger-Parker dominance index are all effective numbers of species of order q = 0, 1, 2 and $\inf$, respectively. (Note that the formula is undefined for q = 1, but its limit $q \to 1$ is). The effective number of species of order q is also often referred to as *Hill numbers*.

Another crucial advantage of the effective number of species of order q is that all indices have the same unit—species or types. We can hence plot the diversity of any community as function of q. The diversity of any given community can be completely described by its *diversity profile* for $0 \leq q \leq \inf$). In this framework, one community can be said to be unconditionally more diverse than another community, only if it is so over the whole range of the diversity profile. If this is not the case (i.e. if the profiles cross) the communities can only be ranked conditionally on q. Take our two imaginary forests above, but add another ten individuals from different species to our spruce monoculture. This forest will now be twice as species-rich (twenty species) compared to the forest with ten equally abundant species (q = 0). Yet, for larger q, it will be *less* diverse than the forest with ten species (because it is still completely dominated by one species). The diversity profiles of these two communities will cross, and we can only decide which is more diverse conditionally on q. An example is give in Fig.3.1.

If a single number should be given, one has to choose the order of q. Some researchers have made the argument that choosing richness as diversity index avoids to have to make a possibly arbitrary decision about how to weight the relative abundances. This is misleading. As shown above, we *have* to choose an order q, richness is no exception but simply the choice of q = 0. I would argue that if any choice should be made, the natural choice is to weight species exactly by their relative abundances (q =1) not to give each species the same weight (q = 0). This is the choice that I have adopted for the chapters included in this thesis.

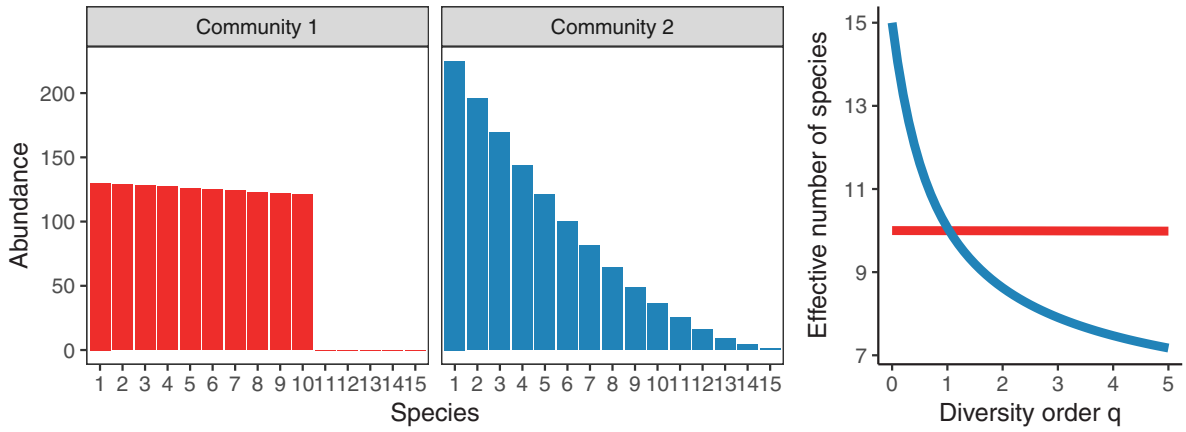

FIGURE 3.1: **Left**: Community with ten species in roughly equal abundances. **Centre**: Community with 15 species in unequal abundances. **Right**: The diversity profiles for both communities for $0 \leq q \leq 5$. Which community is more diverse depends on the order of q.

3.3 A unified framework

Above, I discuss species diversity, functional diversity and phylogenetic diversity as different dimensions of diversity, and how they are potentially related to ecosystem functioning. We can also consider another perspective: while species diversity considers all species to be equally different from each other, functional and phylogenetic diversity weight species by their "relatedness". They are maximized if the species are completely unrelated, either in terms of common ancestry or their functional traits. To describe diversity as completely as possible, we thus need to combine the number of species with information about the relative abundance of species and their relatedness. Such metrics exist and have been popular—e.g. Rao's quadratic entropy (Rao, 1982), which also became a popular measure of functional diversity (Botta-Dukat, 2005). But they are entropies, not diversities. Pavoine et al., (2009) show that many popular metrics could in fact be generalized as phylogenetic entropies of order q, including Faith's PD (q = 0), Rao's Q (q = 1) and Allen et al.'s Hp (q = 2). Based on this, Chao et al., (2010) and Chao et al., (2014b) expanded the framework of Hill numbers to phylogenetic diversity and functional diversity. They refer to their framework as *"attribute*

diversity" where attributes are *"effective number of species"* in the case of species diversity, *"effective number of branches of unit length"* for phylogenetic diversity and the "*effective number of functional species pairs with unit distance"* for functional diversity. As all metrics are effective numbers, they fulfill the doubling property and have the same unit over the full range of q. For phylogenetic diversity, for instance, this means that the diversity of two communities that share no species, and for which the phylogenies are only basally related, is additive.

The metrics can be also seen as "nested". For any given community, the largest diversity value is given by its species richness. If we incorporate information about relative abundance, e.g. if we calculate the effective number of species of order q = 1, the maximum value that can be reached is the species richness—in the case where all species are equally abundant. If we additionally incorporate information about the phylogenetic relatedness, the maximum phylogenetic diversity can be equal to the effective number of species—in the case of a species assemblage were all species are completely unrelated (star phylogeny). The same is true for functional diversity. Effective number of species can thus be seen as a special case of phylogenetic or functional diversity and richness as a special case of the effective number of species. Phylogenetic and functional diversity are the most information-rich metrics of diversity and should probably be preferred.

It is worth noting that the unifying character of the framework described above goes even further. Hill numbers also follow naturally the multiplicative framework of partitioning regional (gamma) diversity into independent alpha (local) and beta (turnover) components, i.e.

$$Gamma\ diversity = Alpha\ diversity * Beta\ diversity \tag{3.2}$$

This unifies a range of popular beta diversity metrics. The Jaccard, the Sørensen, the Horn and Morisita-Horn measures have all been shown to be monotonic transformations of beta diversity based on Hill numbers (Chao et al., 2008, 2012; Chiu et al., 2014; Jost, 2006, 2007). Hence Beta diversities, too, can be plotted as diversity profiles and defined conditionally on the diversity order q.

Another debated question for which this framework proposes a solution is the concept of evenness. It has long been the assumption in ecology that diversity has two components, richness and evenness. From this perception two natural metrics of evenness and "unevenness" can be deducted in the framework of Hill numbers: $Evenness = \frac{Diversity}{Richness}$ and $Unevenness = \frac{Richness}{Diversity}$ (Jost, 2007; Tuomisto, 2012). Only the second decomposition is truly a decomposition into independent components as in the case of the first, evenness is constrained by alpha diversity (Jost, 2007). Note that in both definitions, diversity can be either functional, phylogenetic or species diversity and is contingent on the order q.

3.4 Summary

In my view, metrics that have been used as measure of diversity in the field of diversity and ecosystem functioning research can be broadly characterised into two types. Either they are metrics that can (and should) be transformed into effective number of species (Shannon entropy, Simpson index, Rao's Q, Faith's PD and many more). Or they are metrics that do not actually measure diversity and should not be used as such. The same can probably be said about metrics of beta diversity but I know too little to assess it with determinism. The category of indices that are not diversities includes metrics such as the mean pairwise tip distance MPD or the nearest taxon index NRI (Webb et al., 2008). These metrics are measures of phylogenetic clustering or eveness that can be very useful, e.g. to study species assemblage mechanisms. They are not metrics of diversity. Two communities, one with 2 and one with 200 species both can have the same MPD or NTI.

Diversity is a far more complex concept than we give it credit for. But today, a unified and rigorous framework of diversity exists. If the field of biodiversity and ecosystem functioning takes the concept of diversity seriously, there is no excuse not to fully adopt this framework, and to abandon metrics that are not part of it.

3.5 Estimating diversity in real ecosystems

In most experimental biodiversity and ecosystem functioning studies we manipulate the number of species explicitly and thus do not need to measure it. Yet, there has been a recent interest in studying the effects of diversity in real ecosystems, where the number of species is not manipulated but observed—e.g. Duffy et al., (2016). In studies on natural microbial biodiversity, diversity always has to be estimated, even in experimental settings. This brings one of the fundamental questions of ecology into the field of biodiversity and ecosystem functioning—the question of how to estimate diversity from incomplete samples. The literature on this topic is vast (Magurran, 2004; Magurran and J., 2011) and a comprehensive review is beyond the scope of this introduction. What follows is thus only a brief summary.

There have traditionally been three main classes of diversity estimators: parametric estimators, nonparametric estimators and species accumulation curves. **Parametric estimators** rely on assumptions about the underlying species abundance distributions. If we assume that the "real" species abundance distribution follows a given model, we can fit the distribution to the sample data and then estimate distribution parameter as indicator of richness or other aspects of diversity in the classical sense (i.e. rarity, dominance, evenness, etc.). There are many different species abundance distributions and choosing between these, and accurately fitting them to small samples, can be challenging. Different distributions can produce very different estimates and importantly, parametric estimators do not all measure the same aspect of diversity. Even if they do, their absolute values are not very meaningful and not necessarily comparable. The fitted models can also be used to estimate asymptotic species richness but here again, the estimate will depend on the chosen model and the accuracy of the estimate depends on how well the model describes the real distribution. This makes this class of estimators problematic.

Nonparametric estimators do not make any assumptions about the underlying species distribution (hence the name "nonparametric") but estimate asymptotic species richness based on the ratio of low frequency counts. The most common one, the Chao1 estimator gives a lower bound estimate of

species richness (in its simplest form) as $S + \frac{f_1^2}{2*f_2}$ were f_1 is the number of species with exactly one occurrence in the sample (singletons) and f_2 is the number of species with exactly 2 occurrences. Other estimators include the ACE which makes use of the abundance of rare species (defined as e.g. represented by less than ten individuals) or the Jackknife estimator which uses either only singleton abundance, or both single and doubleton abundance.

The third method—using **species accumulation curves**—consist of plotting the number of recorded species as function of the number of recorded individuals. As the form will vary with the order in which the individuals are recorded, the expectation from N random orders is usually computed. The resulting function can then be extrapolated to get an estimate of asymptotic species richness. The more common use, however, is the reverse scenario. Given a number of samples that differ in the number of recorded individuals, rarefaction can be used to compare the diversity. The idea is to subsample the larger samples repeatedly to get an expectation of what the observed number of species would have been in a sample of that size—to allow a fair comparison between the samples. The problem with this approach is that if samples differ in their true diversity, the sample coverage will differ for a given standardized sampling sizes. Sampling 200 trees in a boreal forest plot is likely to reveal most of the species while a sample of the same size in a tropical forest will only reveal a fraction of the species present. Therefore, Chao and Jost, (2012), proposed a method of coverage based rarefaction and extrapolation where samples are compared at equal coverage, not equal size. Coverage here is the fraction of detected species in the samples, and can be estimated with a coverage estimator that is related to the non-parametric estimators described above.

So far I have discussed ways of estimating species richness. Yet, as discussed above, we might not want to estimate richness as estimate of diversity, but rather effective number of species of higher orders. Chao et al., (2014a), developed a method of rarefaction and extrapolation based on sample completeness for a set of Hill numbers (q = 0, 1, 2, >2). In 2015, Chao et al. extended that framework to a continuous order of q for $0 \leq q \leq 3$. This allows to estimate diversity profiles from sample data. For any given order of q, the rarefaction and extrapolation method also allows to easily judge whether the

sample is sufficient to estimate asymptotic diversity (whether the rarefaction curve reaches an asymptote). This often reveals another advantage of using the effective number of species of order $q = 1$ or higher. While it is almost impossible to get a precise and unbiased estimate of species richness, especially in mega-diverse communities (insects, microbes, tropical trees, etc.) it is often possible—with reasonable sampling effort—to get a precise and unbiased estimate of the effective number of species of order $q = 1$ or higher (Chao et al., 2015a). Finally, (Chao and Jost, 2015) developed methods for rarefaction and extrapolation with phylogenetic richness that have been extended to phylogenetic diversities of order q by Hsieh and Chao, (2017).

Note that I have not touched upon a range of related aspects. For example, to correctly estimate diversity it is not only necessary to extrapolate from the sample at hand, but the sample itself must be generated with care, as discussed in Gotelli and Colwell, (2001). I have also only discussed the case where we have information about species abundance, not only occurrence. Many of the methods that estimate species richness (but not diversity) have related derivations that can work with occurrence data. In addition, estimating diversity naturally comes with uncertainty. Most methods mentioned above also have derivations that allow for the estimation of the variance around the point estimate. In biodiversity and ecosystem functioning, where diversity is used as independent variable, the variance around the estimate is often ignored (as it is also in this thesis). There are statistical methods that allow for variance in the predictor variable (Errors-in-variables models). Another possibility could be to rank samples by their estimated diversity and to assign the same rank to those samples the diversities of which cannot be distinguished with any statistical certainty. Then, rank correlations can be used instead.

In summary, as diversity has mostly been highly controlled in biodiversity-ecosystem functioning experiments, estimating it has not been an issue. Even more recent studies that investigated biodiversity-ecosystem functioning in natural ecosystems (Gamfeldt et al., 2013; Maestre et al., 2012; Paquette and Messier, 2011) usually measured both diversity and ecosystem functioning in moderately small plots, where a complete census is feasible. However, the field has evolved, and is moving towards scenarios where we will have to

estimate diversity from sampling data—see e.g. Duffy et al., (2016). This is true for microbial ecosystems, for studies in other megadiverse ecosystems such as tropical rainforests and coral reefs, but also if larger scales should be studied. In these cases, the question of how to estimate diversity precisely and with little bias becomes a pre-requirement for studying its consequences for ecosystem functioning. Many methods exist but need to be adopted.

3.6 Estimating diversity in microbial ecosystems

In any given ecosystem, microbial diversity in general, and bacterial diversity in particular, is undoubtedly greater than the macrobial diversity. Yet exactly *how* diverse natural bacterial communities are is still unknown, and the estimates vary widely. The first estimates come from whole DNA re-association studies that assume that the re-association kinetics of a mixture of full genomes will depend on the heterogeneity of the community DNA, and hence on the number of species it contains. The methods assume a species definition of 70% DNA hybridization and some average genome size. Based on such calculation, Torsvik et al., (1990), estimated the number of species in $1\,\mathrm{gram}$ of dry soil to be around 4×10^3 Later studies, incorporating assumptions about relative abundance distributions concluded, with otherwise similar methods, that the diversity should rather be in the range of 8×10^6 species (in $10\,\mathrm{gram}$ of soil, Gans et al., 2005). In the meantime, using parametric models assuming a lognormal distribution of bacterial abundances, Curtis et al., (2002), gave estimates of 6×10^3 to $3.8 \times 10^4\,\mathrm{species}\ \mathrm{g}^{-1}$ soil and not more than $4 \times 10^6\,\mathrm{species}$ globally. Sogin et al., (2006), using non-parametric diversity estimators on 454-pyrosequencing data, estimated the diversity of bacteria in $1\,\mathrm{L}$ seawater (close to deep sea hydrothermal vents) to be between $6 \times 10^3\,\mathrm{and}\ 2 \times 10^4\,\mathrm{species}$. Finally, in one of the more recent estimates, Locey and Lennon, (2016) use scaling laws between species richness and the number of individuals, as well as the methodology proposed by Curtis et al., (2002). The authors conclude that global bacterial richness lies in the ballpark of $10 \times 10^{12}\,\mathrm{species}$—which incidentally is also the estimated number of stars in our Galaxy. According to this study, the global oceans harbor $10 \times 10^{10}\,\mathrm{species}$ and the human gut alone some $10 \times 10^6\,\mathrm{species}$. The wide range and partial inconsistencies of the estimates reveal how difficult it is to

estimate bacterial diversity with high confidence, even in small samples. The reason for this are varied and I discuss some of them below.

3.6.1 The bacterial and archeal species concept

One of the most fundamental problems with bacterial and archeal species diversity is the definition of "species". The "gold standard" has been a species definition that is based on DNA-DNA hybridization which indirectly quantifies the similarity of whole genomes (Kim et al., 2014). A 70% threshold for species delimitation has been proposed based on this method and is still recommended (Rosselló-Mora and Amann, 2001; Tindall et al., 2010). Yet this method—while useful for bacterial taxonomy—cannot be used to determine the number of species in a sample. Today, the most common definition of bacterial and archeal species is based on the similarity of the 16S rRNA gene sequence. The 16S rRNA gene encodes the small subunit of the bacterial ribosomal protein. Because of the fundamental importance of the ribosomal protein (it performs the translation of messenger RNA to proteins) it is highly conserved and can be found in all living species. In 1994, Stackebrandt and Goebel determined that *"organisms that have less than 97.0% sequence homology will not re-associate to more than 60%"*. This has become the most widely used threshold since and is probably closest to a universally used species concept for bacteria. Yet, it is imperfect. While 16S rRNA has been validated as phylogenetic marker it was also shown that the threshold for species delimitation varies (mostly between 1 and 3%, Barraclough et al., 2009). Kim et al., (2014), suggest that the best unique threshold would be 98.65% and Větrovský and Baldrian, (2013), caution that 42% of genera have a 16S rRNA sequence similarity of below 97%. On the other hand, some species contain two copies of 16S rRNA that differ by more than 3% within their genome (Rosselló-Mora and Amann, 2001). Therefore, the field of microbial ecology has for the most part adopted a pragmatic solution and used the term of Operational Taxonomic Unit (OTU) instead of species. The most widely used threshold for OTUs remains 97% albeit new methods advocate a data-driven OTU clustering (Mahé et al., 2015), or to avoid OTU clustering all-together (Callahan et al., 2016).

3.6.2 Massive parallel sequencing

The largest practical problem for the estimation of bacterial and archeal species richness is their sheer abundance. As stated in an editorial in Nature Review Microbiology ("Microbiology by numbers" 2011), on a global scale *"we have only sequenced* 10×10^{-22} *% of the total DNA . . . This means that the fraction of microbial diversity that we have sampled to date is effectively zero"*. At small local scales, estimates of bacterial and archeal abundance vary but usually range around 10×10^9 cells g^{-1} soil or sediment Frossard et al., (2016) and $10 \times 10^5 - 10 \times 10^6$ cells ml^{-1} in freshwater or the ocean (own data). Hence, even for a single gram of soil or millilitre of water it has traditionally been unfeasible to sample the community to sufficient depth—or anything close to that. Massive parallel sequencing, often referred to as Next Generation Sequencing or NGS, has changed the rules. Today's technology can generate 10×10^7 or even 10×10^8 reads per run. This enormous sampling effort can be targeted to just the gene of interest by *amplicon sequencing* (so called as the region of interest is extracted and *amplified* by PCR prior to the sequencing). Hence, at least in theory, and for the smaller samples, a complete census is nowadays in the realm of the possible. This has revolutionized the field of microbial ecology. Yet, while this partly alleviates the problem of sufficient sampling, another problem arises: as of today, the NCBI 16S ribosomal RNA targeted loci project (`https://www.ncbi.nlm.nih.gov/refseq/targetedloci/`), which contains high quality consensus sequences and annotations from a range of contribution databases, lists 17968 sequences. This is less than 2% of the estimated number of species inhabiting the human gut alone and a vanishingly small fraction of the total estimated global species diversity. Therefore, and especially if we sample environmental samples, the majority of species that we will uncover are previously unknown. But how do we know that the sequences are from new species? The methodology that is needed to reach such high sampling intensity (amplicon sequencing) is known to produce artificial sequences of two types: 1) chimeric sequences—which are a "mix" of two (or more) genuine sequences that were combined during PCR amplification—and 2) sequencing errors, most commonly the misidentification of a base during sequencing. This will typically generate a large amount of rare sequences that make it hard to distinguish

between genuine rare sequences from unknown species and artificial rare sequences.

Advancements in bioinformatics allow to counter this problem to some extent. Chimera filtering algorithms calculate the probability that sequences are generated by a mix of two sequences of higher abundance and often remove up to 80% or more of the unique sequences in a dataset. Other algorithms filter out sequences that are likely to contain sequencing errors. As Illumina gives a quality score for each base call (the probability that any given base-call is correct—based how unambiguous the signal is) it is possible to calculate the number of expected errors in any given sequence and to filter out sequences that contain more than a certain number of expected errors (Edgar, 2013). As a final step, sequences are clustered to OTUs (mostly at 97% identity) so that sequences containing only a few errors are clustered together with the (more abundant) genuine sequences. OTUs that contain only a single sequence are excluded. This reduces the amount of spurious sequences significantly and increases the likelihood that observed sequences represent real biological variants. Yet, it is also conservative as in any natural sample we would typically expect to detect a large fraction of the species only a single time, if at all. It also makes it difficult to apply non-parametric richness estimators to sequencing data. Non-parametric estimators rely on the ratio of doubletons to singletons. If singletons are excluded they cannot be applied, but if singletons are kept, an inflated singleton count will lead to a dramatic overestimation of bacterial richness. Chiu and Chao, (2016), suggest to solve this problem by replacing the singleton count with an estimated singleton count based on the ratio of quadruplicons, triplicons and duplicons. How effective this is has still to be evaluated, and if it is, it is only a solution for the estimation of taxon diversity, not phylogenetic diversity or beta diversity.

3.6.3 Robust estimation of bacterial diversity

The problems outlined above hinder a reliable estimate of bacterial and archeal richness in all but the least diverse communities. However, it has been suggested (Bent and Forney, 2008), and shown in simulations (Haegeman et al., 2013), that it is possible to reliably estimate the effective number of species of order $q = 1$ or higher, and with high certainty. As I have argued, this might

also be the more relevant metric to estimate in the context of biodiversity - ecosystem functioning research. Even more relevant might be the estimation of phylogenetic diversity (of order $q \geq 1$). Phylogenetic diversity has large advantages in the context of microbial communities. First, it is not necessary to decide upon a clustering threshold for OTUs. Instead, if sequences are stringently denoised using the power of newly developed algorithms (Callahan et al., 2016), a phylogenetic tree can be constructed with all unique sequences. This does not inflate the diversity estimate as sequences that are closely related will contribute very little unique phylogenetic information. Second, it is rarely possible to calculate functional diversity as the vast majority of species have no cultured representatives and most are supposedly unculturable. Therefore the option to calculate species-based functional diversity does not exist in most cases, and phylogenetic diversity is the only option. Naturally, phylogenetic diversity is only a good proxy of functional diversity if the relevant traits are phylogenetically conserved. This seems to be the case for some traits but less so for others (Martiny et al., 2013; Philippot et al., 2010), and horizontal gene transfer can decouple functional capacity from phylogenetic identity.

Both phylogenetic diversity and diversity estimates based on Hill numbers in general face unique challenges in the case of microbial communities. With the current NGS technology, the whole 16S rRNA gene is too long to be sequenced. The most common technology, Illumina MiSeq, can sequence a fragment length of 300 bp (2x150 paired-end) which covers only 20% of the full length. Therefore a shorter fragment spanning 1 or 2 hypervariable regions (so called hypervariable loops for the secondary structure of 16S rRNA) is sequenced. While phylogenies constructed from these shorter sequences reflect phylogenies based on the full sequences well (Jeraldo et al., 2011), they are not perfect. Beyond that, constructing phylogenies with tens of thousands of sequences is challenging. Impressively fast algorithms have been developed (Price et al., 2009) but these, too, come at the price of a certain reduction in accuracy.

Regarding diversities based on Hill numbers in general, they rely on accurate abundance information for the different sequences or OTUs. This is challenging to achieve due to both biological and technical biases. Fundamentally there is no 1:1 correspondence between the number of 16S copies and the number of cells as bacteria have a varying number of 16S rRNA copies in their genome. Větrovský and Baldrian, (2013), show that over half the analysed bacterial genomes genomes have > 5 copies and, moreover, cells can at times have multiple genomes (4 - 18 and up to 100 in cyanobacterial resting stages). This can both over- and underestimate diversity: if a species that is abundant in the sample also has multiple 16S rRNA copies, the dominance of this species will be overestimated and diversity will be underestimated. On the other hand, if a moderately abundant species possesses an excessive number of 16S rRNA copies, the assemblage will seem more even than it is and diversity will be overestimated. Kembel et al., (2012) showed that the number of copies is phylogenetically conserved to a certain degree and suggests to estimate the copy numbers for species where it is unknown—by extrapolating from known species that are phylogenetically related. Yet, the set of species for which this information is known is small and taxonomically biased, wherefore extrapolation can be haphazardous.

PCR biases pose an additional problem. These arise if during PCR amplification of 16S rRNA genes some templates get amplified more than others—either by chance or because of slight differences in the primer affinities—and especially if the template concentrations are low (Kennedy et al., 2014). In addition, different primer sets targeting different regions of the 16S rRNA are all taxonomically biased to some extent (Shakya et al., 2013). Potentially most challenging for the estimation of diversity and species composition in natural samples is what has been called relic DNA—DNA from dead cells that has not been degraded yet. Carini et al., (2016), showed that relic DNA is abundant in soil (40% of 16S genes on average) and can bias estimates of richness (overestimated by 14% on average) and community similarity as well as the relative abundance of taxa.

3.6.4 Summary

Accurately estimating the community composition and diversity of natural bacterial and archeal communities at the level of species used to be impossible. With massive parallel sequencing technologies it is no longer so, but challenges remain. Some are likely to be at least partly resolved in the future. Multiple large sequencing projects, including the Human Microbiome Project, the Earth Microbiome Project, the TARA Global Ocean Sampling and the Ocean Sampling Day Consortium all contribute to a much better coverage of the existing diversity and hence to larger and better reference databases. More sophisticated algorithms will allow to retrieve accurate sequences with higher confidence Callahan et al., (2016) and cluster sequences into biological meaningful OTUs of variable breadth Mahé et al., (2015). New sequencing technologies, as developed by Pacific BioSciences, will allow for much longer reads and sequencing of the full length 16S rRNA genes (although today at the cost of high error rates and biased coverage). As the number and taxonomic breadth of full genome sequences increases, it might become more realistic to take differential copy numbers into account. More importantly, however, studies on mock communities indicate that today's technology performs surprisingly well (D'Amore et al., 2016; Shakya et al., 2013). Especially in experimental settings—where the absolute relative abundance might be less important than the shift in relative abundances in response to an experimental treatments—the methods prove to be robust. The field should now embrace the call to use more robust and more informative metrics of microbial community diversity which allow robust estimation of absolute levels of microbial diversities that are comparable across studies, sample types and biomes.

Section 4

Multifunctionality

4.1 Multifunctionality metrics and their limitations

The idea of multifunctionality was put forward by different groups of researchers independently. The the first was (Duffy et al., 2003), who studied the effects of grazer diversity in seagrass beds. The authors found, that *"only the most diverse grazer assemblage maximized multiple ecosystem properties simultaneously"* - yet without attempting to quantify multifunctionality. Hector and Bagchi, (2007) were the first in the field to talk explicitly about 'multifunctionality', and the authors quantified it *"as the proportion of ecosystem processes with different most-important species"* (as determined by multiple regression models). This approach has been called the "species turnover approach". An alternative way of measuring multifunctionality was suggested by Gamfeldt et al., (2008), who quantified multifunctionality directly, as the probability of a species assemblage to sustain all functions above a certain threshold—set to 50% of the maximum observed functioning. Zavaleta et al., (2010) expanded on this approach by quantifying the number of functions sustained above a certain threshold and explored several threshold levels (30, 40 and 50%). To avoid the need to decide upon a certain threshold level, (Byrnes et al., 2014) proposed the "multiple-threshold approach". The authors suggested to quantify the slope of the relationship, between diversity and the number of functions above the threshold, for all threshold levels—and to plot the slope against the threshold level. The authors also derived a range of multifunctionality metrics from the slope pattern. Using a differnt approach, Mouillot et al., (2011) and Maestre et al., (2012) calculated multifunctionality as the average of standardized function values (the "averaging approach"),

and recently (Dooley et al., 2015) proposed to examine multifunctionality in a multivariate modelling framework.

Each method has its limitations, as summarised by Byrnes et al., (2014). The method of jointly considering multiple single functions (Duffy et al., 2003) does not assess multifunctionality quantitatively. The turnover approach (Hector and Bagchi, 2007) does propose a way of quantification but falls short of quantifying multifunctionality directly (the approach rather assesses whether different species are statistically associated to different processes and to what extent). Moreover, the method only considers positive effects on multifunctionality. It also requires a rather large amount of data to fit the necessary multiple linear models assessing each species' effects on each function. The recently proposed multivariate modelling framework (Dooley et al., 2015) shares some of these limitations. While it allows for a very detailed assessment of the effect of each species, their relative abundances and their interactions, it too does not quantify multifunctionality directly. The multivariate modelling framework is also very data hungry. Suggestions to get around this problem can require difficult and possibly arbitrary decisions (e.g. to model interactions between functional groups, not species. For that, species need first to be classified into functional groups). Other suggestions (setting the interaction coefficient to be equal for all two-species interactions) significantly reduce the information content. These might be part of the reasons for why the approach has not been adopted in new studies, even though it likely is a powerful tool under certain circumstances. One example could be an applied agricultural context where the effect of a small number of target species on a defined set of functions is examined.

All variants of the threshold approach (Gamfeldt et al., 2008; Zavaleta et al., 2010) and the average approach (Maestre et al., 2012; Mouillot et al., 2011) fulfill the criterion of quantifying multifunctionality directly. Yet, both have other limitations: the average approach effectively sets all functions equal so that the higher performance in one function can be offset by the low performance in a completely unrelated function. Even more problematic, in my opinion, is that the number of functions does not feature in the resulting metric. The same average value can result from averaging two functions or twenty. The threshold approach avoids both problems. By counting the

number of functions above one or multiple thresholds it 1) linearly increases with the number of functions considered and 2) counts each function independently, so that unrelated functions cannot "cancel out" each other. Yet, it, too is problematic. For one, we only quantify *if* a function is above the threshold but we do not know by *how much*. Choosing the threshold implies an arbitrary choice of threshold levels. And while the multiple-threshold approach overcomes this last problem, it does not provide a single value and has a range of fundamental flaws that are discussed in detail in **Chapter IV**. In the multiple-threshold approach, the slope value depends on the number of functions and number of species considered (a purely mathematical effect), and the slope pattern depends on the method of standardization of function values. Even more importantly, in order to interpret the slope pattern in a meaningful way, it has to be compared to the expected pattern under some null-model. Yet we show in **Chapter IV** that this null-expectation is very variable—which makes a comparison with the null model haphazardous.

4.1.1 A way forward?

While maybe focussing too much on solely the weaknesses of the current methods, the previous section still makes it clear that, at the moment, there is no metric of multifunctionality that satisfies all our expectations. To go forward from there it is important to articulate what our expectations for a better metric are. Based on what has been discussed in the literature, the metric should fulfill the following criteria:

- It should quantify multifunctionality directly
- It should avoid the averaging of unrelated functions
- It should be sensitive to a continuous change in function values

I would add the following necessary properties to the list:

- It should grow with the number of functions maximized
- It should be insensitive to the addition or removal of a perfectly correlated function

- It should behave predictably so that that deviations from a null expectation can detected and quantified.

This list is somewhat ad-hoc and certainly incomplete. The suggestion that follows below is a collection of preliminary thoughts that will need to be developed further.

The question of how many functions a given community performs can be reformulated to ask "how *diverse* is the functioning". This highlights parallels to the question of how to measure diversity in a community. The naive suggestion would be to simply count the number of functions and calculate the functional richness. Yet, this ignores at what level the functions are performed. Therefore, the natural choice would be to weight the functions by the relative level at which they are performed and calculate the *effective number of functions of order* q. For that, we simply replace the relative abundances of species by the relative performance of functions in the calculation. Note that the relative abundances here have to be calculated twice: once to bring functions on a common scale (by dividing by the observed local maximum or some other maximum value that should be taken as reference—based on the literature or some larger scale observations), and a second time to calculate the relative proportions of standardized function values. This also allows us to calculate functional profiles equivalent to diversity profiles. They convey information about how many functions are performed in total ($q = 0$), how many functions are performed effectively ($q = 1$), and how many functions are dominant ($q = 2$). Yet, so far we only incorporate information about the relative performance of functions, but not their absolute level (where the "absolute" level here refers to the standardized function values). Two communities with the same number of functions performed at the same relative levels (all at 80% of the maximum in community A and all at 20% of the maximum in community B) will result in the same value of effective multifunctionality. One possibility to feature in information about absolute levels of the functions, could be to multiply the effective number of functions by the average level at which the functions are performed. This would equate a community that performs five effective functions on average at 80% with a community that performs ten effective functions on average at 40%.

A problem is that while this metric grows with the number of functions considered, it grows by the same amount if perfectly correlated functions are added. Hence the metric can be artificially inflated by adding more "functions" that are little more than different metrics for the same underlying variable. To avoid this, we can take the 'relatedness' of functions into account. Chao et al., (2014b) developed and index of functional Hill numbers of order q, which calculates the effective number of equally abundant and (functionally) equally distinct species. It is calculated taking into account species pairwise distances and their relative abundances. Replacing species by functions, and quantifying the distance between functions as one minus the absolute correlation coefficient, should result in the *effective number of uncorrelated functions*. This metric should be invariant to the addition of perfectly correlated functions. If we now want to adjust the effective number of uncorrelated functions by their average performance, we should calculate the weighted mean of the performance vector, such that each function is weighted proportional to its average correlation to all other functions.

Some potential problems come to mind. First, we use the absolute correlation coefficient between functions and hence equate positive and negative correlations. It is not quite clear to me at this stage what the implications of this are, and whether it is a problem. Yet, it will need to be considered. The larger problem is the question whether correlations between any two functions are invariant. This is not necessarily the case. Functions could be linked for some species but not for others and hence the overall correlation will depend on the species composition. The correlation between functions could also be conditional on biotic or abiotic variables—like species interactions and climate. Finally, the correlation can be spurious and not reflect an underlying causal link between the functions. In light of these potential problems, it might be better to not take correlations among functions into account at all, but impose the responsibility on the investigator to only include functions that can conceivably vary independently. Yet, this is no formal criterion and will require possibly arbitrary judgement calls.

In summary, I think that the proposed metric has potential but needs to be explored further. Before any new approach should be proposed, the numerical behaviour should be thoroughly tested—to avoid proposing a metric

that turns out to be uninterpretable. More fundamentally, the scientific community needs to agree what we do, and do not, understand when it comes to multifunctionality, and what we expect a metric of multifunctionality to achieve.

Thus far, I have avoided a definition of "function". What constitutes a function is somewhat poorly defined. It is important to realize that this problem cannot, and will not, be solved by a metric of multifunctionality. While phylogenetic diversity circumvents to some extent the need of a species definition for bacteria, multifunctionality cannot do the same for the definition of "function". Therefore, a measure of multifunctionality will always be only as informative as the single functions it summarizes. Chances are, that because of this fundamental limitation, the concept of multifunctionality will be more useful and less contested under circumstances where the functions of interest are clear. This might be in an applied context where a set of services should be maximized, or in a basic research context, if the available expert knowledge is sufficient to define what set of functions is of particular interest.

Section 5

Concluding Remarks

The literature discussing the importance of biodiversity on ecosystem functioning is large and I am likely to be unaware of its majority. The same is true for the relevant ecological concepts —which are too diverse for me to apprehend them all. In the light of this, it feels contemptuous to make generalizations, and any attempt is deemed to be either trivial or unjust. What follows is my personal take based on the insights I have gained thus far.

Demonstrating the importance of biodiversity is arguably the driver behind the question of whether diversity is important for ecosystem functioning. The research area surged in popularity as reaction to the ongoing human-induced extinction crisis. The imminent threat of losing species is one reason why *species richness*, above other metrics of diversity, has been the focus of the majority of research efforts. It is also the reason why the question is of paramount importance. Yet, as I have argued above, the focus on the number of species as predictor variable for ecosystem functioning is problematic. In itself, across all possible species combinations, it is a far too wide concept to be a useful predictor for any ecosystem process. Accordingly, species are rarely ever sampled from the pool of all possible species, but rather from a small pool of phylogenetically and/or functionally closely related species. Within this small subset of 'sensible' species combinations, species are usually combined at equal abundances—which in turn is only one of an infinite number of possible combinations of relative abundances for the same species richness value. Thus I argue that while species richness has been the "official" independent variable in the majority of studies, it never actually was. This mismatch, between what we say we manipulate and what we actually

do manipulate, is problematic. To be able to predict the consequences of local species loss (or gain!) it is unlikely to help if we attempt to generalize over all experiments that correlated the number of species *per se*. Acknowleding the complexity of the diversity concept, and using more information-rich metrics, is a necessary step in the right direction. But it does not overcome the fundamental problem.

The application of biodiversity and ecosystem functioning research to natural microbial ecosystems illustrates the challenge. Fig.5.1 shows the phylogenetic tree published in 2016 byHug et al., (2016) in a article appropriately titled *"A new view of the tree of life"*. Opisthokonta, the phylogenetic group comprising all animals (metazoa) as well as fungi, is a single branch on the tree. Any environmental sample containing a natural bacterial and archeal community can contain representatives from any number of the overwhelming majority of remaining branches. In **Chapter III**, we found 8187 bacterial and archeal OTUs in total, with a median of 2870 OTUs. Putting aside the species concept and the problems of estimating microbial richness, what does knowing these numbers tell us about the functioning of the community? For context, the number of possible species combinations from a species pool that large—for a community consisting of not more than 30 species—is already larger than the *estimated number of atoms in the universe* ($>10 \times 10^{80}$; the number of combination for the median sample is $>10 \times 10^{2300}$).

No part of this argument implies that biodiversity (in any form) cannot be important for ecosystem functioning. It has been proven beyond doubt that, in certain contexts, species assemblages containing more species are e.g. more productive or more stable over time than species assemblages containing only a subset of the same species. But the quest for a general (or even function- or trophic-level specific) biodiversity-ecosystem functioning relationship is, in my view, a lost cause. Knowing the diversity of an assemblage will newer, on its own, be a good predictor for ecosystem functioning. If that was the case, my allotment should be highly productive. Sadly it is not. I am not implying either, that the experiments performed thus far have been fruitless. They were not Eisenhauer et al., (2016) and Tilman et al., (2014). Yet, the main conclusion—that the collective evidence proves that diversity *as such* is

important for ecosystem functioning, or ecosystem multifunctionality—does not hold.

It is crucial to add that this has no implication for the urgency of preserving biodiversity. The biodiversity of life on earth, the result of 4 billion years of evolution, is earth's most amazing property. For all we know, it sets our planet apart from the remaining known universe. Protecting the ecosystems and organisms with which we share the same planet is a moral imperative. From a utilitarian perspective, one virtue of the field of biodiversity and ecosystem functioning has been to demonstrate the myriads of ways in which human society is deeply dependent on nature. We therefore should always apply the cautionary principle and avoid irreversible changes. As human impact is unavoidable, we need the best possible knowledge base to make evidence-based and informed decisions. Research in ecology is crucial to provide this knowledge. To be reliable, it must be as rigorous as possible. This thesis hopes to provide some small steps in the right direction.

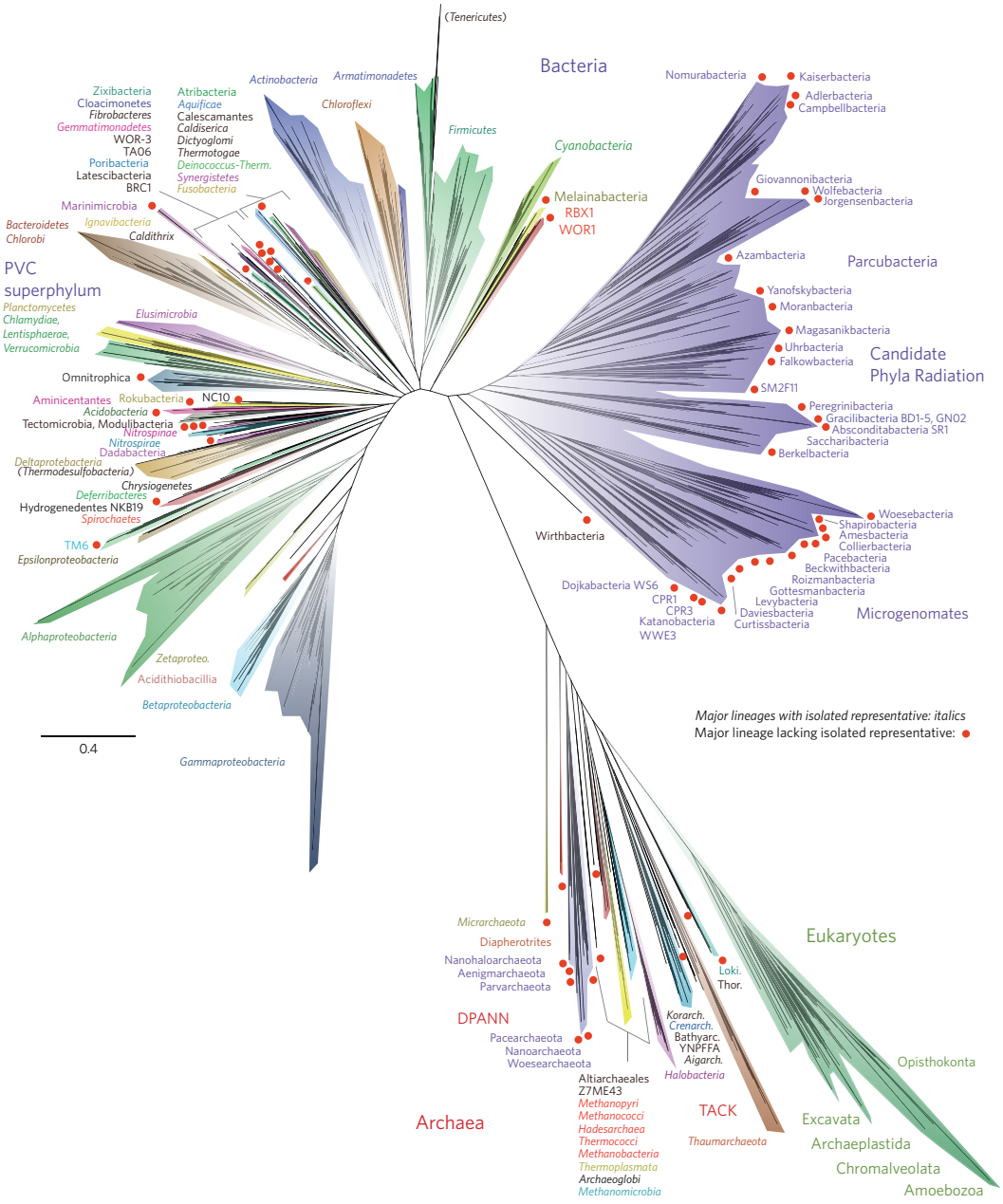

FIGURE 5.1: A new view of the tree of life, from Hug et al., (2016). Note that all animals and fungi fit on a single branch (Opisthokonta).

Bibliography

Alexandrou, M A et al. (2014). "Evolutionary relatedness does not predict competition and co-occurrence in natural or experimental communities of green algae". In: *Proceedings of the Royal Society B: Biological Sciences* 282.1799, p. 20141745.

Andersson, B et al. (2014). "Nitrogen fixation in shallow-water sediments: Spatial distribution and controlling factors". In: *Limnology and Oceanography* 59.6, pp. 1932–1944.

Angelini, C et al. (2015). "Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes". In: *Proceedings of the Royal Society B: Biological Sciences* 282.1811, p. 20150421.

Barraclough, T G et al. (2009). "Inferring evolutionarily significant units of bacterial diversity from broad environmental surveys of single-locus data". In: *Biology Letters* 5.3, pp. 425–428.

Bell, G (1991). "The ecology and genetics of fitness in Chlamydomonas III. Genotype-by-environment interaction within strains". In: *Evolution* 45.3, pp. 668–679.

Bengtsson, J (1998). "Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function". In: *Applied Soil Ecology* 10.3, pp. 191–199.

Bent, S J and L J Forney (2008). "The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity". In: *The ISME Journal* 2.7, pp. 689–695.

Botta-Dukat, Z (2005). "Rao's quadratic entropy as a measure of functional diversity based on multiple traits". In: *Journal of Vegetation Science* 16.5, pp. 533–540.

Burley, H M et al. (2016). "Primary productivity is weakly related to floristic alpha and beta diversity across Australia". In: *Global Ecology and Biogeography* 25.11, pp. 1294–1307.

Byrnes, J E K et al. (2014). "Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions". In: *Methods in Ecology and Evolution* 5.2, pp. 111–124.

Cadotte, M W (2013). "Experimental evidence that evolutionarily diverse assemblages result in higher productivity." In: *Proceedings of the National Academy of Sciences USA* 110.22, pp. 8996–9000.

— (2015). "Phylogenetic diversity and productivity: gauging interpretations from experiments that do not manipulate phylogenetic diversity". In: *Functional Ecology* 29.12, pp. 1603–1606.

Cadotte, M W et al. (2008). "Evolutionary history and the effect of biodiversity on plant productivity". In: *Proceedings of the National Academy of Sciences USA* 105.44, pp. 17012–17017.

Cadotte, M W et al. (2009). "Using Phylogenetic, Functional and Trait Diversity to Understand Patterns of Plant Community Productivity". In: *PloS one* 4.5, e5695.

Cadotte, M W et al. (2011). "Beyond species: functional diversity and the maintenance of ecological processes and services". In: *Journal of Applied Ecology* 48.5, pp. 1079–1087.

Callahan, B J et al. (2016). "DADA2: High-resolution sample inference from Illumina amplicon data". In: *Nature Methods* 13.7, pp. 581–583.

Cardinale, B J et al. (2011). "The functional role of producer diversity in ecosystems." In: *American Journal of Botany* 98.3, pp. 572–592.

Cardinale, B J et al. (2012). "Biodiversity loss and its impact on humanity". In: *Nature* 486.7401, pp. 59–67.

Cardinale, B J et al. (2015). "Further re-analyses looking for effects of phylogenetic diversity on community biomass and stability". In: *Functional Ecology* 29.12, pp. 1607–1610.

Carini, P et al. (2016). "Relic DNA is abundant in soil and obscures estimates of soil microbial diversity". In: *Nature Microbiology* 2, p. 16242.

Chao, A and L Jost (2012). "Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size." In: *Ecology* 93.12, pp. 2533–2547.

— (2015). "Estimating diversity and entropy profiles via discovery rates of new species". In: *Methods in Ecology and Evolution* 6.8, pp. 873–882.
Chao, A et al. (2008). "A Two-Stage Probabilistic Approach to Multiple-Community Similarity Indices". In: *Biometrics* 64.4, pp. 1178–1186.
Chao, A et al. (2010). "Phylogenetic diversity measures based on Hill numbers". In: *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 365.1558, pp. 3599–3609.
Chao, A et al. (2012). "Proposing a resolution to debates on diversity partitioning". In: *Ecology* 93.9, pp. 2037–2051.
Chao, A et al. (2014a). "Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies". In: *Ecological Monographs* 84.1, pp. 45–67.
Chao, A et al. (2014b). "Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers". In: *Annual Review of Ecology, Evolution, and Systematics* 45.1, pp. 297–324.
Chao, A et al. (2015a). "Rarefaction and extrapolation of phylogenetic diversity". In: *Methods in Ecology and Evolution* 6.4, pp. 380–388.
Chao, A et al. (2015b). "Unveiling the species-rank abundance distribution by generalizing the Good-Turing sample coverage theory". In: *Ecology* 96.5, pp. 1189–1201.
Chiu, C H and A Chao (2016). "Estimating and comparing microbial diversity in the presence of sequencing errors". In: *PeerJ* 4.6, e1634.
Chiu, C H et al. (2014). "Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers". In: *Ecological Monographs* 84.1, pp. 21–44.
Christensen, N L et al. (1996). "The report of the Ecological Society of America committee on the scientific basis for ecosystem management". In: *Ecological applications* 6.3, pp. 665–691.
Curtis, T P et al. (2002). "Estimating prokaryotic diversity and its limits." In: *Proceedings of the National Academy of Sciences USA* 99.16, pp. 10494–10499.
D'Amore, R et al. (2016). "A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling". In: *BMC Genomics* 17.1, p. 2937.

Darnis, G et al. (2012). "Current state and trends in Canadian Arctic marine ecosystems: II. Heterotrophic food web, pelagic-benthic coupling, and biodiversity". In: *Climatic Change* 115.1, pp. 179–205.

Díaz, S and M Cabido (2001). "Vive la différence: plant functional diversity matters to ecosystem processes". In: *Trends in Ecology & Evolution* 16.11, pp. 646–655.

Doak, D F et al. (1998). "The statistical inevitability of stability-diversity relationships in community ecology". In: *The American Naturalist* 151.3, pp. 264–276.

Dooley, A et al. (2015). "Testing the effects of diversity on ecosystem multifunctionality using a multivariate model". In: *Ecology Letters* 18.11, pp. 1242–1251.

Duffy, J E (2002). "Biodiversity and ecosystem function: the consumer connection". In: *Oikos* 99.2, pp. 201–219.

— (2009). "Why biodiversity is important to the functioning of real-world ecosystems". In: *Frontiers in Ecology and the Environment* 7.8, pp. 437–444.

Duffy, J E et al. (2003). "Grazer diversity effects on ecosystem functioning in seagrass beds". In: *Ecology Letters* 6.7, pp. 637–645.

Duffy, J E. et al. (2014). "Ecology of Seagrass Communities". In: *Marine community ecology and conservation*. Ed. by Mark D Bertness et al. Sunderland: Palgrave Macmillan Sinauer Associates. Chap. 12, pp. 271–292.

Duffy, J E et al. (2016). "Biodiversity enhances reef fish biomass and resistance to climate change". In: *Proceedings of the National Academy of Sciences USA* 113.22, pp. 6230–6235.

Edgar, R C (2013). "UPARSE: highly accurate OTU sequences from microbial amplicon reads." In: *Nature Methods* 10.10, pp. 996–998.

Eisenhauer, N et al. (2016). "Biodiversity–ecosystem function experiments reveal the mechanisms underlying the consequences of biodiversity change in real world ecosystems". In: *Journal of Vegetation Science* 27.5, pp. 1061–1070.

Faith, D P (1992). "Conservation evaluation and phylogenetic diversity". In: *Biological Conservation* 61.1, pp. 1–10.

Frossard, A et al. (2016). "Flow Cytometric Assessment of Bacterial Abundance in Soils, Sediments and Sludge". In: *Frontiers in Microbiology* 7.903.

Gamfeldt, L et al. (2008). "Multiple Functions Increase The Importance Of Biodiversity For Overall Ecosystem Functioning". In: *Ecology* 89.5, pp. 1223–1231.

Gamfeldt, L et al. (2013). "Higher levels of multiple ecosystem services are found in forests with more tree species. Nature Communications 4: 1340". In: *Nature Communications* 4, p. 1340.

Gamfeldt, L et al. (2015). "Marine biodiversity and ecosystem functioning: what's known and what's next?" In: *Oikos* 124.3, pp. 252–265.

Gans, J et al. (2005). "Computational improvements reveal great bacterial diversity and high metal toxicity in soil." In: *Science* 309.5739, pp. 1387–1390.

Gotelli, N J and R K Colwell (2001). "Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness". In: *Ecology Letters* 4.4, pp. 379–391.

Grace, J B et al. (2007). "Does species diversity limit productivity in natural grassland communities?" In: *Ecology Letters* 10.8, pp. 680–689.

Gravel, D et al. (2011). "Experimental niche evolution alters the strength of the diversity-productivity relationship". In: *Nature* 469.7328, pp. 89–92.

Griffin, J N et al. (2009a). "Functional diversity predicts overyielding effect of species combination on primary productivity". In: *Oikos* 118.1, pp. 37–44.

Griffin, J N et al. (2009b). "Spatial heterogeneity increases the importance of species richness for an ecosystem process". In: *Oikos* 118.9, pp. 1335–1342.

Griffin, J N et al. (2013). "Effects of predator richness on prey suppression: a meta-analysis". In: *Ecology* 94.10, pp. 2180–2187.

Haegeman, B et al. (2013). "Robust estimation of microbial diversity in theory and in practice". In: *The ISME Journal* 7.6, pp. 1092–1101.

He, J-Z et al. (2009). "Linking soil bacterial diversity to ecosystem multifunctionality using backward-elimination boosted trees analysis". In: *Journal of Soils and Sediments* 9.6, pp. 547–554.

Hector, A and R Bagchi (2007). "Biodiversity and ecosystem multifunctionality." In: *Nature* 448.7150, pp. 188–190.

Hill, M O (1973). "Diversity and Evenness: A Unifying Notation and Its Consequences". In: *Ecology* 54.2, pp. 427–432.

Hooper, D U et al. (2005). "Effects of biodiversity on ecosystem functioning: a consensus of current knowledge". In: *Ecological Monographs* 75.1, pp. 3–35.

Hooper, D U et al. (2012). "A global synthesis reveals biodiversity loss as a major driver of ecosystem change". In: *Nature* 486.7401, pp. 105–108.

Houlahan, J E et al. (2017). "The priority of prediction in ecological understanding". In: *Oikos* 126.1, pp. 1–7.

Hsieh, T C and A Chao (2017). "Rarefaction and Extrapolation: Making Fair Comparison of Abundance-Sensitive Phylogenetic Diversity among Multiple Assemblages". In: *Systematic Biology* 66.1, pp. 100–111.

Hug, L A et al. (2016). "A new view of the tree of life". In: *Nature Microbiology* 1.5, p. 16048.

Hughes, A R et al. (2008). "Ecological consequences of genetic diversity". In: *Ecology Letters* 11.6, pp. 609–623.

Huston, M A (1997). "Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity". In: *Oecologia* 110.4, pp. 449–460.

Isbell, F et al. (2011). "High plant diversity is needed to maintain ecosystem services". In: *Nature* 477.7363, pp. 199–202.

Isbell, F et al. (2015). "Biodiversity increases the resistance of ecosystem productivity to climate extremes." In: *Nature* 526.7574, pp. 574–577.

Jeraldo, P et al. (2011). "On the suitability of short reads of 16S rRNA for phylogeny-based analyses in environmental surveys". In: *Environmental Microbiology* 13.11, pp. 3000–3009.

Jost, L (2006). "Entropy and diversity". In: *Oikos* 113.2, pp. 363–375.

— (2007). "Partitioning diversity into independent alpha and beta components." In: *Ecology* 88.10, pp. 2427–2439.

Kembel, S W et al. (2012). "Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance." In: *PLoS Computational Biology* 8.10, e1002743.

Kennedy, K et al. (2014). "Evaluating Bias of Illumina-Based Bacterial 16S rRNA Gene Profiles". In: *Applied and Environmental Microbiology* 80.18, pp. 5717–5722.

Kim, M et al. (2014). "Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes". In: *International Journal of Systematic and Evolutionary Microbiology* 64.2, pp. 346–351.

Lavorel, S and E Garnier (2002). "Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail". In: *Functional Ecology* 16.5, pp. 545–556.

Lefcheck, J S et al. (2015). "Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats". In: *Nature Communications* 6, p. 6936.

Locey, K J and J T Lennon (2016). "Scaling laws predict global microbial diversity". In: *Proceedings of the National Academy of Sciences USA* 113.21, pp. 5970–5975.

Loreau, M (2000). "Biodiversity and ecosystem functioning: recent theoretical advances". In: *Oikos* 91.1, pp. 3–17.

Loreau, M and A Hector (2001). "Partitioning selection and complementarity in biodiversity experiments". In: *Nature* 412.6842, pp. 72–76.

Loreau, M. et al. (2002). *Biodiversity and ecosystem functioning, synthesis and perspectives.* First edition. Biodiversity and ecosystem functioning, synthesis and perspectives. Oxford: Oxford University Press, p. 294.

Loreau, M et al. (2003). "Biodiversity as spatial insurance in heterogeneous landscapes." In: *Proceedings of the National Academy of Sciences USA* 100.22, pp. 12765–12770.

MacArthur, R H (1965). "Patterns of species diversity". In: *Biological reviews* 40.4, pp. 510–533.

Maestre, F T et al. (2012). "Plant Species Richness and Ecosystem Multifunctionality in Global Drylands". In: *Science* 335.6065, pp. 214–218.

Magurran, A E (2004). *Measuring biological diversity*. Malden, Ma: Blackwell Pub. ISBN: 978-0632056330.

Magurran, A E. and McGill B J. (2011). *Biological diversity : frontiers in measurement and assessment*. Oxford New York: Oxford University Press. ISBN: 978-0199580675.

Mahé, F et al. (2015). "Swarm v2: highly-scalable and high-resolution amplicon clustering". In: *PeerJ* 3.2, e1420.

Martiny, A C et al. (2013). "Phylogenetic conservatism of functional traits in microorganisms". In: *The ISME Journal* 7.4, pp. 830–838.

Meyer, S T et al. (2016). "Effects of biodiversity strengthen over time as ecosystem functioning declines at low and increases at high biodiversity". In: *Ecosphere* 7.12, e01619.

"Microbiology by numbers" (2011). In: *Nature reviews. Microbiology* 9.9, pp. 628–628.

Mora, C et al. (2011). "Global Human Footprint on the Linkage between Biodiversity and Ecosystem Functioning in Reef Fishes". In: *PLoS Biology* 9.4, e1000606.

Mouillot, D et al. (2011). "Functional Structure of Biological Communities Predicts Ecosystem Multifunctionality". In: *PloS one* 6.3, e17476.

Mouquet, N et al. (2012). "Ecophylogenetics: advances and perspectives". In: *Biological Reviews* 87.4, pp. 769–785.

Narwani, A et al. (2013). "Experimental evidence that evolutionary relatedness does not affect the ecological mechanisms of coexistence in freshwater green algae." In: *Ecology Letters* 16.11, pp. 1373–1381.

Naughton, H R et al. (2015). "Phylogenetic distance does not predict competition in green algal communities". In: *Ecosphere* 6.7, art116.

Newbold, T et al. (2015). "Global effects of land use on local terrestrial biodiversity". In: *Nature* 520.7545, pp. 45–50.

O'Connor, M I et al. (2017). "A general biodiversity–function relationship is mediated by trophic level". In: *Oikos* 126.1, pp. 18–31.

Paquette, A and C Messier (2011). "The effect of biodiversity on tree productivity: from temperate to boreal forests". In: *Global Ecology and Biogeography* 20.1, pp. 170–180.

Pavoine, S et al. (2009). "Hierarchical partitioning of evolutionary and ecological patterns in the organization of phylogenetically-structured species assemblages: application to rockfish (genus: Sebastes) in the Southern California Bight". In: *Ecology Letters* 12.9, pp. 898–908.

Petchey, O L and K J Gaston (2002a). "Extinction and the loss of functional diversity". In: *Proceedings of the Royal Society B: Biological Sciences* 269.1501, pp. 1721–1727.

— (2002b). "Functional diversity (FD), species richness and community composition". In: *Ecology Letters* 5.3, pp. 402–411.

— (2006). "Functional diversity: back to basics and looking forward". In: *Ecology Letters* 9.6, pp. 741–758.

Petchey, O L et al. (2004). *How do Different Measures of Functional Diversity Perform?*, pp. 847–857.

Philippot, L et al. (2010). "The ecological coherence of high bacterial taxonomic ranks". In: *Nature reviews. Microbiology* 8.7, pp. 523–529.

Pimm, S L (1984). "The complexity and stability of ecosystems". In: *Nature* 307.5949, pp. 321–326.

Price, M N et al. (2009). "FastTree: computing large minimum evolution trees with profiles instead of a distance matrix." In: *Molecular biology and evolution* 26.7, pp. 1641–1650.

Rao, C R (1982). "Diversity and dissimilarity coefficients: a unified approach". In: *Theoretical population biology* 21.1, pp. 24–43.

Roger, F et al. (2012). “Genetic Diversity and Ecosystem Functioning in the Face of Multiple Stressors”. In: *PloS one* 7.9, e45007.

Rosselló-Mora, R and R Amann (2001). “The species concept for prokaryotes”. In: *FEMS Microbiology Reviews* 25.1, pp. 39–67.

Schindler, D E and M D Scheuerell (2002). “Habitat coupling in lake ecosystems”. In: *Oikos* 98.2, pp. 177–189.

Schulze, Ernst-Detlef and Harold A. Mooney (1993). *Biodiversity and ecosystem function*. Ecological studies (Berlin). Berlin: Springer Verlag, p. 525.

Shakya, M et al. (2013). “Comparative metagenomic and rRNA microbial diversity characterization using archaeal and bacterial synthetic communities. - PubMed - NCBI”. In: *Environmental Microbiology* 15.6, pp. 1882–1899.

Simmons, J P et al. (2011). “False-Positive Psychology”. In: *Psychological Science* 22.11, pp. 1359–1366.

Sogin, M L et al. (2006). “Microbial diversity in the deep sea and the underexplored "rare biosphere"”. In: *Proceedings of the National Academy of Sciences USA* 103.32, pp. 12115–12120.

Solan, M. et al. (2009). “Biodiversity-ecosystem function research and biodiversity futures: early bird catches the worm or a day late and a dollar short?” In: *Biodiversity, ecosystem functioning, & human wellbeing*. Ed. by Shahid Naeem et al. Oxford: Oxford University Press. Chap. 3, pp. 30–45.

Srivastava, D S et al. (2012). “Phylogenetic diversity and the functioning of ecosystems”. In: *Ecology Letters* 15.7, pp. 637–648.

Stackebrandt, E and B M Goebel (1994). “Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology”. In: *International Journal of Systematic and Evolutionary Microbiology* 44.4, pp. 846–849.

Tilman, D et al. (1997a). “Plant diversity and ecosystem productivity: theoretical considerations.” In: *Proceedings of the National Academy of Sciences USA* 94.5, pp. 1857–1861.

Tilman, D et al. (1997b). “The Influence of Functional Diversity and Composition on Ecosystem Processes”. In: *Science* 277.5330, pp. 1300–1302.

Tilman, D et al. (2012). “Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory”. In: *Proceedings of the National Academy of Sciences USA* 109.26, pp. 10394–10397.

Tilman, D et al. (2014). “Biodiversity and Ecosystem Functioning”. In: *Annual Review of Ecology, Evolution, and Systematics* 45.1, pp. 471–493.

Tindall, B J et al. (2010). "Notes on the characterization of prokaryote strains for taxonomic purposes". In: *International Journal of Systematic and Evolutionary Microbiology* 60.1, pp. 249–266.

Torsvik, V et al. (1990). "High diversity in DNA of soil bacteria." In: *Applied and Environmental Microbiology* 56.3, pp. 782–787.

Tuomisto, H (2012). "An updated consumer's guide to evenness and related indices". In: *Oikos* 121.8, pp. 1203–1218.

Tylianakis, J M et al. (2008). "Resource Heterogeneity Moderates the Biodiversity-Function Relationship in Real World Ecosystems". In: *PLoS Biology* 6.5, e122.

van der Plas, F et al. (2016). "Biotic homogenization can decrease landscape-scale forest multifunctionality". In: *Proceedings of the National Academy of Sciences USA*, p. 201517903.

Venail, P et al. (2015). "Species richness, but not phylogenetic diversity, influences community biomass production and temporal stability in a re-examination of 16 grassland biodiversity studies". In: *Functional Ecology* 29.5, pp. 615–626.

Větrovský, T and P Baldrian (2013). "The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses". In: *PloS one* 8.2, e57923.

Vila, M et al. (2007). "Species richness and wood production: a positive association in Mediterranean forests". In: *Ecology Letters* 10.3, pp. 241–250.

Villéger, S et al. (2008). "New Multidimensional Functional Diversity Indices For A Multifaceted Framework In Functional Ecology". In: *Ecology* 89.8, pp. 2290–2301.

Wardle, D A (2016). "Do experiments exploring plant diversity–ecosystem functioning relationships inform how biodiversity loss impacts natural ecosystems?" In: *Journal of Vegetation Science* 27.3, pp. 646–653.

Wardle, D A and O Zackrisson (2005). "Effects of species and functional group loss on island ecosystem properties". In: *Nature* 435.7043, pp. 806–810.

Wardle, D A et al. (1997). "The Influence of Island Area on Ecosystem Properties". In: *Science* 277.5330, pp. 1296–1299.

Webb, C O et al. (2008). "Phylocom: software for the analysis of phylogenetic community structure and trait evolution". In: *Bioinformatics* 24.18, pp. 2098–2100.

Yachi, S and M Loreau (1999). "Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis." In: *Proceedings of the National Academy of Sciences USA* 96.4, pp. 1463–1468.

Zavaleta, E S et al. (2010). "Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity". In: *Proceedings of the National Academy of Sciences USA* 107.4, pp. 1443–1446.

Zhu, Y et al. (2000). "Genetic diversity and disease control in rice." In: *Nature* 406.6797, pp. 718–722.