Microbial Community Ecology and Biotic Processes at the Aquatic/Terrestrial Interface

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SUMMARY

Rivers and their adjacent riparian zones are locations of high levels of biodiversity and are well known for their enhanced rates of important biogeochemical processes. Despite their small total area, rivers contribute disproportionally to regional carbon fluxes and riparian zones are hotspots of terrestrial denitrification. Microorganisms drive these biogeochemical processes as well as serve as the basis of brown food webs and contribute to physical processes such as sediment flocculation and soil aggregation. Despite the importance of microbial communities in rivers and riparian systems, they are relatively understudied in comparison to other riverine organisms.

This doctoral work investigates microbial community structure and function at the aquatic/terrestrial interface. First, a theoretical work based on the newly proposed concept of microbial community coalescence explores the potential consequences of environmental mixing on lotic and riparian microbial community structure. This work takes a catchment-scale perspective of microbial community assembly across ecosystem boundaries. Next, results of a field study conducted across nine rivers in the UK are presented, providing insight about the influence of chemical, hydrological and spatial drivers on sediment fungal community structure. This provides a sub-catchment scale view of lotic fungal diversity. The final chapter details results of an experimental study investigating the influence of collembolans, ubiquitous soil organisms, on the production of the greenhouse gas N_2O. This work explores the effects of biotic-scale processes on ecosystem functioning.

We reviewed field studies investigating environmental mixing processes and found evidence that environmental mixing influences microbial community structure in some compartments, such as headwaters and estuaries. The application of the microbial community coalescence concept in rivers may increase the amount of variance explained between observed local communities. Despite a rich body of literature about lotic fungal decomposer communities inhabiting leaf litter, very few studies investigated general fungal diversity. Our investigation of sediment fungal communities revealed highly diverse communities that were differentiated by underlying geology. Hydrological and

chemical variables explained some of the differences between microbial communities, while spatial variables were less important. Finally, we conducted an experimental study to investigate the microbial-driven process of denitrification – an anaerobic nitrogen cycling process that produces N_2 and N_2O, a greenhouse gas. We found the different species of the ubiquitous soil organism Collembola affect the proportion of N_2O that is produced as an end-product of denitrification and that this is related to shifts in soil nitrate concentrations.

Together, this work reports findings from several under-investigated areas of microbial structure and functioning in rivers, soils and across their interface at three different scales. Our results provide insight about patterns of riverine microbial biodiversity through application of a new conceptual framework that may improve explanatory power and through a field investigation that reveals the relative importance of spatial and environmental drivers. Our field investigation was one of the first studies in Europe to apply next-generation sequencing to general fungal communities in rivers. We also provide evidence that denitrification is impacted by the presence of soil microarthropods, organisms with highly diverse communities in riparian zones. As riverine systems are simultaneously vital for ecosystem function and highly threatened by anthropogenic activity, there is an urgent need for fundamental knowledge of lotic biodiversity patterns and their relationship with function to inform conservation and restoration efforts.

ZUSAMMENFASSUNG

Flüsse und ihre angrenzenden Ufer sind Zonen hoher lokaler Biodiversität, deren gesteigerte biogeochemische Prozesse gut untersucht sind. Trotz ihrer kleinen Gesamtfläche tragen Flüsse überproportional zum lokalen Kohlenstofffluss bei, während die Uferzonen bekannte Hotspots der terrestrischen Denitrifikation darstellen. Mikroorganismen fördern diese biogeochemischen Prozesse mit denen sie die Grundlage der braunen Nahrungskette bilden und physikalische Prozesse wie die Sedimentausfällung und Bodenaggregation begünstigen. Trotz der großen Bedeutung von mikrobiellen Gemeinschaften in Flüssen und Uferzonen, sind diese im Gegensatz zu anderen Flussorganismen nicht ausreichend erforscht.

Diese Doktorarbeit untersucht die Struktur und Funktion von mikrobiellen Gemeinschaften an der Schnittstelle von Boden und Wasser in Flüssen. Zunächst soll in einer theoretischen Abhandlung auf der Grundlage des neu entworfenen Konzepts der mikrobiellen Gesellschaftskoaleszenz ergründet werden, welche Konsequenzen der Prozess des Mischens von mikrobiellen Lebensgemeinschaften der Ströumungs- und Auengewässer auf deren Strukturierung zur Folge hat. Diese Studie nimmt eine Reservoir-basierte Perspektive ein, zur Betrachtung der Zusammenstellung mikrobieller Lebensgemeinschaft jenseits der Ökosystemgrenzen. Nachfolgend sollen die Ergebnisse einer Feldstudie, die neun Flüsse Großbritanniens einbezieht, präsentiert werden. So soll das Verständnis über den Einfluss von chemischen, hydrologischen und räumlichen Variablen, die die Gesellschaftsstruktur von Sedimentpilzen beeinflussen, gewonnen werden. Dies wird durch eine Sub- Reservoir-basierte Perspektive auf die lotische Pilzdiversität ermöglicht. Das letzte Kapitel dieser Arbeit beschreibt detailiert die Ergebnisse einer experimentellen Studie zur Untersuchung des Einfluss von Springschwänzen (Collembolen) auf die Produtkion des Treibhausgases Distickstoffmonoxid N_2O. Diese Studie erforscht den Effekt von Prozessen der biotischen Ebene auf Ökosystemfunktionen.

Mittels Literatursynthese untersuchten wir den Einfluss der Mischung von mirkobiellen Gesellschaften mitsamt der sie umgebenden Umwelt (Koaleszenz) auf deren Lebensgeimschaftsstruktur. Wir fanden heraus, dass sich die Struktur in bestimmten

Abschnitten, wie Oberwasser und Ästuar, verändert. Die Anwendung des Konzepts der mikrobiellen Gesellschaftskoaleszenz in Flüssen hat das Potenzial, die beobachtete Variabilität zwischen den lokalen Lebensgemeinschaften zu erklären. Obwohl lotische Pilze das Ziel zahlreicher Untersuchungen sind und waren, gibt es nur wenige Studien, die deren generelle Diversität erfoschen. Unsere Studie enthüllte eine diverse Lebensgemeinschaft von lotischen Pilzen, die Blattstreu bewohnten, und deren Lebensgemeinschaftstsruktur von der Geologie weiter differenziert wird. Hydrologische und chemische Variablen erklärten einige der Unterschiede zwischen den mikrobiellen Lebensgemeinschaften, während die räumlichen Variablen weniger informativ waren. Im finale Experiment zur Erforschung der mikrobiell beeinflussten Denitrifikation fokussierten wir uns auf den anaerobsichen Stickstoffzyklus, der das Treibhausgas Distickstoffmonoxid hervorbingt. Dabei fanden wir heraus, dass verschiedene Springschwanzarten, wobei es sich um ubiquitären Bodenorganismen handelt, das Verhältnis von Stickstoff und Distickstoffmonoxid beeinflussen können.

Zusammengefasst bietet diese Doktorarbeit Ergebnisse aus verschiedenen, wenig erforschten Bereichen der Struktur mikrobieller Lebensgemeinschaften und Ökosystemfunktionen in Flüssen, Böden und jenseits ihrer beider Schnittstelle auf drei verschiedenen Ebenen. Unsere Erebnisse ermöglichen neue Einsichten in Muster der flussnahen mikrobiellen Lebensgemeinschaften durch die Anwendung eines neuen Rahmenkonzepts und Felduntersuchungen mit verbesserter Aussagekraft zur Bedeutung von Raum- und Umweltfaktoren. Unsere Feldstudie war eine der ersten ihrer Art in Europa, in der eine neue Generation von Sequenziermethoden zum Einsatz kam, um Pilzlebensgemeinschaften in Flüssen zu charakterisieren. Ebenfalls konnten wir beweisen, dass der Prozess der Denitrifikation durch die Anwesenheit von Bodenmikroarthropoden, Organismen mit sehr diversen Lebensgemeinschaften in flussnahen Zonen, beeinflusst wird. Es besteht ein dringender Bedarf nach fundamentalem Wissen über lotische Biodiversitätsmuster und deren Beziehung zu Ökosystemfunktionen, um die Bemühungen im Naturschutz und –widerherstellung zu verbessern. Unsere flussnahen Systeme sind sowohl lebensnotwendig für die Funktionsfähigkeit unserer Ökosystem als auch gefährdet durch anthropogene Aktivitäten, denen wir nur mit Wissen beikommen können.

THESIS OUTLINE

This thesis is a cumulative work, consisting of four manuscripts that have either been accepted for publication or are ready for submission to a peer-reviewed journal. The general introduction (Chapter 1) provides background, context and research aims for the works herein and the general discussion section (Chapter 6) makes conceptual linkages between the results of the studies. The references for each manuscript follow that manuscript directly, and the references cited in the general introduction and discussion sections have been merged into a common reference section at the end of the thesis.

Chapter 1: General Introduction

Chapter 2: Mansour I, Heppell CM, Ryo M & Rillig MC. (2018). Application of the Microbial Community Coalescence Concept to Riverine Networks. Biological Reviews. doi: 10.1111/brv.12422

Author contributions: All authors contributed to the conceptualization and writing of this review paper.

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Chapter 4: Mansour I, Heppell CM, McKew BA, Dumbrell A, Whitby CB, Veresoglou S, Leung G, Binley AM, Trimmer M & Rillig MC. (in preparation). Deterministic Processes Drive Fungal Community Assembly at the Sub-catchment Scale.

Author contributions: IM, CMH, BAM & CBW designed the study, IM, CMH, BAM, CBW, GL, AMB & MT conducted field and laboratory work, IM, CMH, BAM, AD, CBW, SV & MCR contributed to data analysis and interpretation, IM, CMH, SV and MCR contributed the manuscript

Chapter 5: Mansour I, Arce M, Marhan S, Rillig MC & Veresoglou S. (in preparation). Collembolans Impact Soil Nitrous Oxide Production.

Author contributions: IM, MCR & SV designed the study; IM & SV conducted the microcosm study; IM, MA & SM performed laboratory work; IM, MA & SV analyzed the data, IM, MCR & SV contributed to the manuscript

Chapter 6: General discussion

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1. GENERAL INTRODUCTION

1.1 Microbes at the aquatic/terrestrial interface

The works documented in this thesis are concerned with the structure and function of microbial communities that inhabit rivers and the soils adjacent to them, i.e. riparian zones. Broadly defined, microorganisms are microscopic unicellular or multicellular organisms. Use of the terms ‘microorganism’ or ‘microbial’ here is somewhat narrower, and includes microscopic organisms in the kingdoms Bacteria, Archaea and Fungi. In the context of rivers and riparia, microbial communities drive many fundamental ecosystem processes, such as decomposition, nutrient cycling and soil aggregation; discussed in further detail in section 1.2. They also interact with other biota through trophic interactions (e.g. serving as the basis of the brown food web), mutualisms and parasitism.

1.1.1. Microbial Habitats

Microorganisms inhabit riverine compartments with wide-ranging chemical, hydrological and physical characteristics. Habitats within the river are referred to as lotic habitats. Free-living, particle- and litter-associated microorganisms can be found in the water column, or pelagic habitat, where they are continually, passively moved downstream by stream flow. Microbial communities in benthic (i.e. riverbed) sediments often form complex and highly active biofilms, which are important habitats in headwaters and tidal flats (Battin *et al.* 2009, 2016). Microorganisms also inhabit the hyporheic zone, or those subsurface sediments through which surface water-groundwater exchange occurs. The riparian zone is a unique and diverse terrestrial habitat that is influenced by lotic biota and can be subject to varying degrees of flooding disturbance (Naiman *et al.* 2005; Muehlbauer *et al.* 2014).

1.1.2 Microbial biogeography and drivers of microbial community structure

Riverine microbial diversity has been somewhat well characterized for some microbial groups and habitats, while there is minimal data for others. At the catchment scale, spatial processes have been shown to be an important factor structuring lotic microbial

communities. Some longitudinal catchment-scale studies investigating benthic (Besemer *et al.* 2013) and pelagic (Crump *et al.* 2012; Savio *et al.* 2015) bacterial communities have observed a decrease in biodiversity from headwaters to estuary (although others have not, e.g. Read *et al.* 2015). These studies reported diverse communities including soil-derived taxa in upper reaches and a shift to more typical freshwater taxa in downstream reaches. There is some controversy in the literature regarding the ecological processes driving lotic bacterial diversity patterns, which have been explained by both ecological succession (Read *et al.* 2015) and by the metacommunity paradigms of mass effects and species sorting (Besemer *et al.* 2013; Savio *et al.* 2015). The latter explanation is supported by a study that sampled across the aquatic-terrestrial interface in soils, headwaters and lower reaches and found that 80% of aquatic sequences represented taxa derived from soil and soil water and that the abundance of these taxa increased further downstream (i.e. shifts in community structure rather than replacement) (Ruiz-González *et al.* 2015). Very few studies have investigated general lotic fungal community structure at the catchment scale. Contrary to observations of general bacterial communities, one study in a Japanese river observed fungal richness to increase from headwaters to estuary; however, nestedness was not observed for most fungal groups, indicating replacement of taxa across the longitudinal continuum (Miura and Urabe 2015b). Spatial patterns have also been reported to be significant drivers of fungal community structure in two other studies of East Asian rivers (Liu *et al.* 2015; Yu *et al.* 2017). These findings align with recent river-specific ecological theory that recommends taking a network perspective that explicitly considers the directionality, connectivity and hierarchal structure of rivers to explain lotic biodiversity patterns (Altermatt 2013).

In addition to spatial factors, environmental drivers also play a role in shaping lotic and riparian microbial communities. Water and sediment chemistry (Fierer *et al.* 2007; Savio *et al.* 2015), hydrological regime (Widder *et al.* 2014) and season (Hullar *et al.* 2006; Rubin and Leff 2007) have been shown to influence lotic bacterial diversity. These environmental factors have also been shown to be important for benthic biofilm diversity (including bacteria and other organisms; reviewed by Besemer 2015; Battin *et al.* 2016). Drivers of general lotic fungal diversity are less well resolved, but land use (Miura and Urabe 2015a), water depth (Yu *et al.* 2017) and carbon quantity and quality (Miura and

Urabe 2015b) have been correlated with fungal community structure. The diversity of the functional group of lotic decomposer fungi, the aquatic hyphomycetes, has been better studied and found to be correlated with temperature (Bärlocher *et al.* 2008; Krauss *et al.* 2011), pH (Bärlocher 1987; Krauss *et al.* 2011), acidification and eutrophication (Lecerf and Chauvet 2008). Riparian microbial community structure has been found to be influenced by hydrologic connectivity (Freimann *et al.* 2015), soil moisture, organic matter content, and soil bulk density (Stutter and Richards 2012); soil moisture and organic carbon have also been found to be influential in microcosm and mesocosm experiment simulating flooding (Drenovsky *et al.* 2004; Wilson *et al.* 2011).

1.2 Microbial functions

Microorganisms play key functional roles in rivers and riparian habitats, contributing to carbon and nutrient cycling as well as influencing physicochemical parameters though sediment flocculation (Droppo *et al.* 1997), soil aggregation (Mardhiah *et al.* 2014) and supporting early-successional plant development (Harner *et al.* 2011). These processes are not trivial: despite their small relative surface area, rivers play an important role in the global carbon cycle (Cole *et al.* 2007) and are responsible for a large percentage of global denitrification activity (Trimmer *et al.* 2012).

1.2.1 Carbon cycling

Significant exchange of carbon occurs across the aquatic terrestrial interface. Large amounts of carbon in rivers originates from terrestrial sources (i.e. allochthonous material), estimated at 1.9 Pg carbon per year (Cole *et al.* 2007). Allochthonous leaf litter is directly and indirectly broken down by aquatic hyphomycete fungi (Hieber and Gessner 2002; Krauss *et al.* 2011) in the pelagic, benthic and hyporheic compartments (Danger *et al.* 2012). Freshwater fungal biomass (Langhans *et al.* 2008; Manerkar *et al.* 2008) and sporulation have frequently been positively correlated with leaf breakdown, but the impact of diversity has less frequently been investigated. Leaf mass loss has been positively correlated with increasing diversity of aquatic hyphomycetes some studies (Bärlocher and Corkum 2003; Clivot *et al.* 2014) but not others (Dang *et al.* 2005; Harrop *et al.* 2009; Geraldes *et al.* 2012). Fungal species identity (Bärlocher and Corkum 2003)

and traits (Krauss *et al.* 2011) have been suggested to have a larger impact on leaf decomposition than fungal species richness. From a catchment-scale perspective, dissolved organic carbon sources have been shown to be degraded continuously from headwaters downstream; degradation of more recalcitrant carbon in (typically more well-lit) downstream reaches may be stimulated by priming via autochthonous production of labile carbon (Battin *et al.* 2009).

Carbon can also move from rivers to riparian zones: large quantities of river-derived particulate organic carbon can be delivered to floodplains during flooding events, where bacterial decomposition is important (Robertson *et al.* 1999). Within-stream microbial carbon dynamics are also important: autochthonous microbial production (e.g. by cyanobacteria) can be significant, this carbon is subsequently transferred to higher trophic levels in the food web (Risse-Buhl *et al.* 2012). Factors including light availability and hydrological connectivity have been shown to be important determinants of whether stream metabolism is net heterotrophic (i.e. driven inputs of allochthonous litter) or net autotrophic (i.e. driven by in-stream production) (Rovelli *et al.* 2017).

1.2.2 Nitrogen cycling

Rivers and riparian contribute important fluxes of nitrogen to global N budgets. A myriad of phylogenetically diverse microbial groups contribute to nitrogen cycling processes, including nitrogen removal (via denitrification and annamox) and transformations of inorganic and organic forms of nitrogen (via nitrification, dissimilatory nitrate reduction to ammonium and nitrogen mineralization). Reviewing the plethora of literature relating to riverine nitrogen cycling is beyond the scope of this introduction (but see Helton *et al.* 2011 for a catchment-scale overview), and thus here will focus only on denitrification, which is further investigated in Chapter 5. Denitrification is an anaerobic process involving the stepwise reduction of nitrate (NO_3^-) to the gaseous products nitrous oxide (N_2O) and dinitrogen (N_2). In riparian zones buffering agricultural land, denitrification may serve to lessen the load of nitrate from agricultural runoff entering water bodies (Boz *et al.* 2013). This, along with in-stream denitrification (and annamox; see for example Lansdown *et al.* 2016) are N-removal processes that reduce the amount of bioavailable

nitrogen that could otherwise contribute to eutrophication. However, N_2O, one of the end products of denitrification, is a potent greenhouse gas with a 120 year atmospheric lifetime (Braker and Conrad 2011). Denitrification in riparian zones is an important component of terrestrial N_2O fluxes (Bouwman *et al.* 2013) and riverine denitrification is estimated to contribute 10% of global N_2O emissions (Beaulieu *et al.* 2011). The ratio of N_2O to N_2 produced as a result of denitrification can vary depending on environmental context.

Thus understanding the dynamics of this process is important for the management of agricultural catchments: on one hand it reduces the potential for eutrophication, but on the other hand can produce greenhouse gas emissions. Denitrification is a stepwise process in which oxidized nitrogen species are used as a final electron acceptor for anaerobic microbial respiration. There are at least seven key enzymes involved in the pathway, distributed among various bacterial, archaeal and fungal taxa (Philippot 2002; Maeda *et al.* 2015). Across ecosystems, the key controls on local denitrification rates are substrate availability (i.e. nitrate and carbon), O_2 concentration, pH and temperature, while distal controls on denitrifier community composition include environmental variability, disturbance and predation (Wallenstein *et al.* 2006). In riparian zones and floodplain soils, denitrification activity has been also shown to be influenced by flood pulses (Shrestha *et al.* 2014), soil texture (Pinay *et al.* 2000) and land use (Sgouridis *et al.* 2011), and the activity of denitrifier taxa may be controlled by redox condition (Seo and DeLaune 2010). In rivers, geomorphic controls such as sediment characteristics (Tatariw *et al.* 2013) and hydrologic connectivity (Tomasek *et al.* 2017) have been shown to influence denitrifier community structure. Biotic drivers have also been shown to influence denitrification activity: tubificid worms stimulated denitrification in river sediments in one study (Mermillod-Blondin *et al.* 2004) and N_2O emissions in soils have been shown to shift in the presence of AM fungi (Bender *et al.* 2014) and earthworms (Marhan *et al.* 2015). The biotic controls on denitrifier community structure and activity are not well resolved compared to environmental drivers.

1.2.3 Microbial functions link aquatic and terrestrial ecosystems

The microbial functions discussed in this section are non-exhaustive, but represent those that will be considered in the following chapters. Even within this limited scope of ecosystem functions, several examples emerge illustrating the linkages made by microbial activity between rivers and riparia. Microbial activity in river channels controls the fate of terrestrial-derived carbon: fungal colonization of leaf litter improves palatability for invertebrate shredders (Hieber and Gessner 2002); these fungal also directly compose litter (Krauss *et al.* 2011). Conversely, the microbial activities leading to flocculation of dissolved organic carbon (DOC) could lead to temporary protection from degradation. In heterotrophic streams, terrestrial carbon inputs control carbon availability and thus processes such as denitrification. In riparian soils, mycorrhizal fungi reduce soil erosion by surface water flow (Mardhiah *et al.* 2016), thereby also reducing provisions of soil-derived carbon and microbial taxa. These examples illustrate the importance of integrating aquatic and terrestrial ecology, as several recent works have recommended (Naiman *et al.* 2005; Soininen *et al.* 2015).

1.3 Research Aims

Microbial communities in rivers and riparian zones host high levels of biodiversity and play important roles in numerous ecosystem functions; however, there are many unexplored avenues of microbial community ecology in these habitats. The doctoral work comprising this thesis targets some of these research gaps. Chapters 2 and 3 develop the recently proposed concept of microbial community coalescence. This represents a novel conceptualization of the dynamics of microbial community assembly following environmental mixing and may serve to improve the amount of variability explained between local communities. This concept departs from pre-existing community ecological theories in that it is microbial-specific and explicitly considers shifts in the abiotic/resource environment that often occur following mixing events. The concept was applied to riverine networks (Chapter 2) through discussion of mixing events in rivers and presentation of evidence from a review of field studies. The potential for community cohesion or connectedness to influence the outcome of a microbial community coalescence event was developed in a dispatch paper (Chapter 3). Chapter 4 reports the

findings of a field study investigating benthic sediment fungal communities across sites of differing geology. While lotic decomposer fungi have been widely studied, there are a limited number of studies reporting on general lotic fungal diversity and none that have considered underlying geology as a driver of community structure. This field study was conducted at sites with clay, Greensand and Chalk geology and that lay across a hydrological gradient of baseflow. The effects of spatial and environmental predictor variables on fungal community structure were tested. Finally, Chapter 5 details an experiment exploring the influence of soil biota on denitrification activity. Several recent studies report effects of various fauna on denitrification; however, biotic controls are not well resolved. We conducted a microcosm study using two species of soil microarthropods, Collembola, and tested potential denitrification activity and soil parameters. These four works serve to provide insight about microbial community assembly and ecosystem functioning in rivers, riparian zones and across their boundary.

2. APPLICATION OF THE MICROBIAL COMMUNITY COALESCENCE CONCEPT TO RIVERINE NETWORKS

India Mansour, Catherine M. Heppell, Masahiro Ryo and Matthias C. Rillig

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3. MICROBIAL ECOLOGY: COMMUNITY COALESCENCE STIRS THINGS UP

Matthias C. Rillig and India Mansour

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4. DETERMINISTIC PROCESSES DRIVE LOTIC FUNGAL COMMUNITY ASSEMBLY AT THE SUB-CATCHMENT SCALE

India Mansour, Catherine M. Heppell, Boyd McKew, Alex Dumbrell, Corinne B. Whitby, Stavros Veresoglou, Garwai Leung, Andrew M. Binley, Mark Trimmer, and Matthias Rillig

4.1 Abstract

Despite their essential roles in ecosystem functioning, exceptionally little is known about fungal communities and the ecological processes regulating their structure. This is particularly true for riverine ecosystems, where very few studies have investigated the structure and environmental drivers of general fungal diversity. In this field study, benthic sediment samples and surface water samples were collected seasonally from lowland rivers (Hampshire Avon catchment, UK) underlain by three distinct parent geologies (clay, Greensand and Chalk), across a hydrological gradient of baseflow index ranging from 0.23 to 0.95. Fungal communities were assessed using high-throughput sequencing and community data were analyzed via ordination, variation partitioning and indicator species analysis. We found that distinct fungal communities inhabited the benthic sediments of the differing geologies. Clay sediments were dominated by the yeast *Cryptococcus podzolicus*, the hyphomycete *Pseudeuotium hygrophilum*, Mortierella, and unidentified fungi in the class Sordariomycetes – the latter two also common within Greensand sediments along with seasonal spikes in *Rhizophydium littoreum*, a parasite of green algae. An unidentified fungus from the phylum Ascomycota was numerically dominant at all Chalk sites and across all seasons. Spatial variables explained only a negligible proportion of variance between communities, indicating that environmental and biotic processes drive the differences between the observed fungal communities rather than purely spatial mechanisms (e.g. stochastic processes). Season was a highly significant predictor of community structure ($p=0.005$) and baseflow index explained some of the variance within the fungal community data across seasons. This study demonstrates that deterministic rather than stochastic processes are important for structuring lotic fungal communities, and, for the first time, shows that underlying geology and associated differences in hydrology are drivers of fungal community structure. Since riverine ecosystems are often subject to high levels of natural and

anthropogenic stressors, it is imperative to understand the mechanisms regulating riverine fungal communities before appropriate management options can be suggested.

4.2 Introduction

Fungal communities participate in a variety of important biogeochemical processes and ecosystem functions, including decomposition (Krauss *et al.* 2011), nitrogen cycling (Veresoglou *et al.* 2012; Maeda *et al.* 2015), soil aggregation (Rillig and Mummey 2006) and primary production via influencing plant communities (Bever *et al.* 2010). Despite this, general fungal diversity is understudied, particularly compared to bacterial communities and instead, large research efforts have focused on specific functional groups, such as mycorrhizal fungi in soils. In lotic systems, there has been a particular focus on fungal decomposition of allochthonous leaf litter, carried out by aquatic hyphomycete fungi. A rich body of literature dating back to the 1950s describes this group of fungi, including their distribution (Duarte *et al.* 2016) and environmental transport (Cornut *et al.* 2014; Chauvet *et al.* 2016), their relative role in litter decomposition (Gulis and K. Suberkropp 2003; Pascoal and Cássio 2004; Das *et al.* 2007), as well as the influence of leaf litter diversity (Bärlocher and Graça 2002; Bärlocher and Corkum 2003; Nikolcheva and Barlocher 2005; Das *et al.* 2008), warming (Bärlocher *et al.* 2008; Ferreira and Chauvet 2011; Fernandes *et al.* 2012; Duarte *et al.* 2013) and pollution (Baudoin *et al.* 2008; Duarte *et al.* 2008, 2015; Lecerf and Chauvet 2008; Clivot *et al.* 2014) on the structure and function of these communities. It is logical that a large effort has gone into deepening our understanding of aquatic hyphomycetes: they carry out the critical ecosystem process of litter decomposition and therefore influence riverine metabolism, and are easy to study using traditional techniques due to their large and characteristic conidia. However, studies into other functional groups and general riverine fungal diversity are sparse and therefore little is known about the structure and function of lotic fungal communities beyond this functional group.

Despite the broad application of molecular techniques in studies of freshwater bacteria (Thompson *et al.* 2017) and terrestrial fungi, general freshwater fungal communities remain largely understudied. The most recent review describing the distribution and taxonomy of general aquatic fungi was based mainly on culture-based and microscopy

studies (Shearer *et al.* 2007). Key findings were that the highest fungal diversity is present in temperate regions and that among fungal phyla, Ascomycetes are common, while Basiodiomycetes are relatively rare in freshwater environments. However, it is likely that majority of these studies vastly underestimated the true degree of fungal diversity present in their samples because of technical limitations. A more recent review (Tornwall *et al.* 2015) of riverine biodiversity highlights the paucity of studies investigating lotic microbial communities. We could identify only three studies applying molecular methods to investigate patterns of benthic fungal diversity; all study locations were located in East Asia (Miura and Urabe 2015b; Liu *et al.* 2015; Yu *et al.* 2017). These studies provide initial evidence about general lotic fungal communities; however, there are no studies in European rivers and knowledge about the composition and drivers of these communities remains limited.

Water chemistry is often reported in field studies of lotic fungi; however, it is typically used as a predictor variable for ecosystem processes, such as litter decomposition rates, rather than fungal community structure. Bärlocher (Bärlocher and Corkum 2003) infers from his own data as well as an earlier study (Suberkropp and Chauvet 1995) that low levels of inorganic nutrients limit aquatic hyphomycete diversity. However, neither study conducted a statistical analysis testing the robustness of this correlation, nor applied molecular methods to identify fungal species, thereby including only a subset of total fungal diversity. A manipulative study of biofilm N and P concentrations found no relationship between nutrient limitation and fungal community composition (Hoellein *et al.* 2010). The quantity and quality of carbon resources has been reported to influence fungal community structure (Bärlocher and Graça 2002; Miura and Urabe 2015a). Despite limited and contradictory evidence for the influence of water and/or sediment chemistry on lotic fungal communities, there is evidence that elevated nutrient concentrations affect bacterial communities in rivers (Rubin and Leff 2007) and microbial (including fungal) communities in soils (Leff *et al.* 2015), thus further investigations are needed to resolve controls of chemical parameters on freshwater fungal community structure.

Hydrological regime may also influence riverine fungal communities. A recent review provides evidence for the influence of such characteristics as hydrological connectivity and habitat determination on biodiversity (Rolls *et al.* 2017). Studies across a range of different lotic biota have reported significant effects of hydrological environmental predictors (Tornwall *et al.* 2015); this trend is also observed in studies of stream microbial communities (Zeglin 2015). Water depth (Yu *et al.* 2017) and longitudinal position along the river (Miura and Urabe 2015b) have been reported to influence fungal communities; however, vertical connectivity has not previously been studied. Upwelling groundwater could affect benthic fungal communities via provision of groundwater-derived fungal taxa and alteration of surface water chemistry.

In this study we aimed to address the knowledge gap in general riverine fungal biodiversity and investigate the drivers of these communities. Sampling sites were underlain by three different parent materials and were located across a hydrological gradient. We hypothesized that unique fungal communities would inhabit differing geologies and that both spatial processes and environmental drivers, including water chemistry, carbon resource quantity/quality and proportion of groundwater contributing to stream discharge, would influence general lotic fungal community structure.

4.3 Materials and Methods

4.3.1 Site description

The Hampshire Avon catchment is located in southwest England. The underlying catchment geology is largely Cretaceous Chalk (86%) with outcroppings of Upper Greensand (13%) and some areas of Late Jurassic Kimmeridge Clay (1%); three sampling locations were selected with each of these underlying geologies (Figure 4.1). Land use in this area is largely agricultural, including horticulture and livestock production. The sites comprise a hydrological gradient of baseflow, a parameter that describes the long-term ratio of groundwater contribution to total stream discharge (calculated on an annual basis). The parameter can be well-approximated by a model based on the hydrology of soil types, BFIHOST (Bloomfield *et al.* 2009). The BFIHOST values for our study sites were accessed from the Flood Estimation Handbook. The clay

sites are characterized by low base flow values (BFIHOST = 0.234-0.635), minimal growth of autochthonus vegetation, diverse and abundant riparian vegetation (including Ash, Buckthorn, Hawthorne and oak trees; see Table S1), and the surrounding land is typically used for grazing of domestic livestock. The Chalk sites are characterized by high base flow values (BFIHOST = 0.838-0.953), large amounts of autochthonous vegetation growth in spring and summer (20-34% coverage), minimal riparian vegetation (resulting from removal) and the surrounding land-use is arable farmland. The Greensand sites were intermediate between Chalk and clay by these metrics. See Allen *et al.* (2014) and Heppell *et al.* (2017) for more detailed sites descriptions.

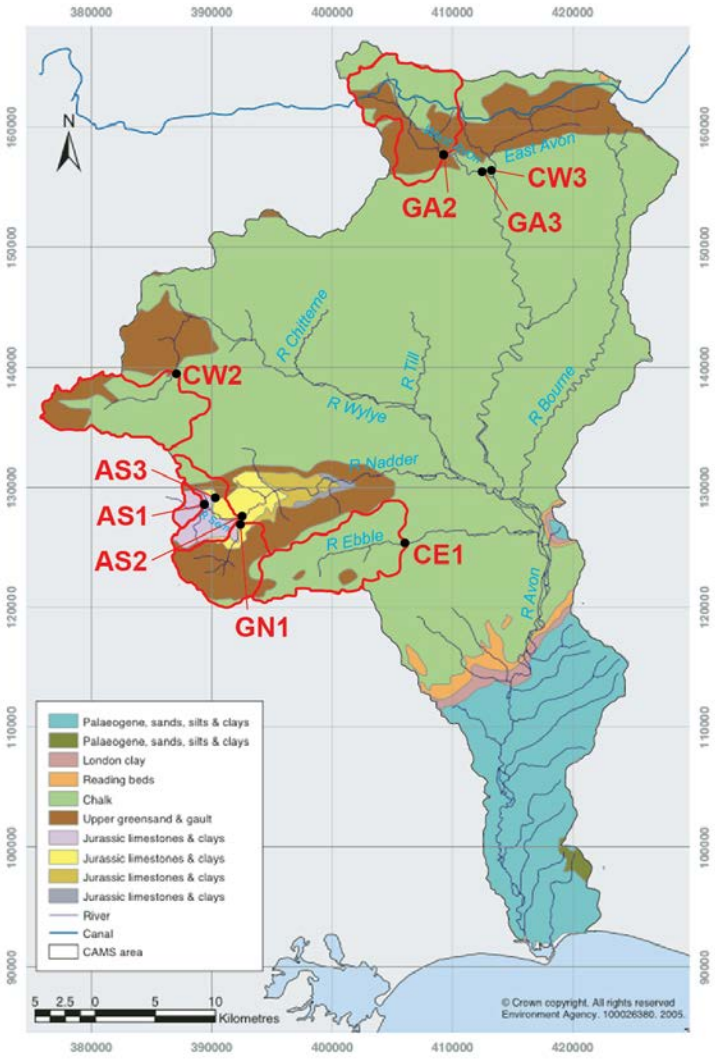

Figure 4.1 Sampling locations in the Hampshire Avon catchment
CXX – Chalk sites, GXX – Greensand sites, AXX – clay sites

4.3.2 Sampling

Benthic sediment samples were collected seasonally (February, April, August and November) in 2013 from the Rivers Wylye and Ebble (Chalk), Nadder and Avon (Greensand) and Sem (clay). The sites comprise a hydrological gradient, with the highest volumes of groundwater inputs occurring at the Chalk sites (i.e. highest BFIHOST values) and the lowest at the clay sites. Samples were collected from the top 5cm of benthic sediment by hand with 9cm (internal diameter) Perspex cores. Approximately 2g was preserved cryogenically at -150°C immediately on-site for subsequent molecular analysis until transferred to long-term storage at -80°C. At two of the sites, additional samples were collected from vegetated and marginal bank sediments. Riparian tree diversity was surveyed along both banks of 30m of the reach proximal to the sediment sampling location. Surface water samples were collected for analysis of water chemistry, passed through 0.2 µm syringe filters, and stored frozen.

4.3.3 Water and sediment chemistry

Anions and cations were measured in surface water samples and various carbon compounds were quantified from sediment core samples. Anions and cations were measured using a Dionex Ion Chromatogram ICS3000 (Thermo Scientific, Waltham, MA, USA) with an AS18 column and KOH and MilliQ water eluents against a 7 point calibration curve of reference standards from 0-200 µmol L^{-1}. Chlorophyll-a and phaeopigments were extracted from a freeze-dried and homogenized sub-core using the cold methanol method (Stal *et al.* 1984). Sub-cores were dried at 60°C and homogenized for the measurement of colloidal carbohydrates (extracted at 30°C and 100°C) and extracellular polymeric substances (extracted in 30% and 70% ethanol) following the methods described by Hanlon *et al.* (Hanlon *et al.* 2006). Total carbon (TC) and total organic carbon (TOC) were quantified from known weights of sediment from dried and homogenized sub-cores. To drive off organic carbon, 2M HCl was added to the TOC sample. Both TC and TOC were measured at 900°C in a Shimazdu TOC-V (Shimazdu, Kyoto, Japan) with solid sample module.

4.3.4 Molecular work

DNA was extracted from 0.25g wet weight sediment subsamples using the PowerSoil ® DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, CA, USA) following instructions from the manufacturer and subsequently stored at -20°C until further use. An amplicon library was prepared for MiSeq analysis. The ITS2 region was targeted using primers fITS7 (5' – GTGARTCATCGAATCTTTG – 3') and ITS4 (5' – TCCTCCGCTTATTGATATGC – 3') (Ihrmark *et al.* 2012) with P5 and P7 (respectively) Illumina Nextera Index overhang adapters in a 25 µL PCR reaction consisting of 12.5 µL RedTaq® Readymix™ (Sigma Aldrich, St. Louis, MO, USA), 1 µL each of 10µm primer solutions, 10 µL sterile water and 0.5 µL DNA. The thermal cycling conditions were as follow: 1 cycle of 95°C for 5m, 32 cycles of 95°C for 30s, 53°C for 30s, and 72°C for 45s and a final extension at 72°C for 10m. Thermal cycling was carried out using a Veriti 96-well thermal cycler (Applied BioSystems, Foster City, CA, USA). PCR cleanup was conducted using Agencourt AMPure XP® (Beckman Coulter, High Wycombe, UK) magnetic SPRI beads. Briefly, 20 µL AMPure beads were added to each well and mixed by pipetting. After a 5m incubation, the plate was placed on a magnetic stand and supernatant removed and discarded once cleared. The beads were washed twice with 80% ethanol, then allowed to air dry for 10m. The plate was removed from the stand and 52.5 µL of 10mM Tris was added to each well and mixed well. Following a 2 min incubation at RT and then a 2 min incubation on the magnetic stand, 50 µL of supernatant was transferred to a new 96-well plate. Indexing PCR was performed using the Nextera XT DNA Library Preparation kit (Illumina, San Diego, CA, USA) in a 50 µL reaction consisting of 5 µL DNA, 5 µL each of indexing primers, 25 µL RedTaq® Readymix™ and 10 µL sterile water. The thermal cycling conditions were as follows: 1 cycle of 95°C for 3m, 8 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s and a final extension at 72°C for 5m. A second DNA cleanup was performed using the same method as above, with reagent volumes adjusted for the larger volume of PCR product: 56 µL of AMPure® beads, 37.5 µL of Tris buffer, and 25 µL of the final supernatant was transferred to a clean 96 well plate. DNA was quantified using a Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoScientific, Waltham, MA, USA) on a FLUOstar Omega plate reader (BMG Labtech, Ortenberg, Germany). Based on the measured DNA concentrations, an

equimolar pool of indexed PCR products was prepared for sequencing on the MiSeq platform. Sequencing was performed on an Illumina MiSeq System using a 600 cycle MiSeq reagent Kit v3 by the Earlham Institute, formerly The Genome Analysis Centre (Norwich, UK).

4.3.5 Bioinformatics

Sequence reads were quality trimmed using Sickle (Joshi and Fass 2011), error corrected within SPAdes (Nurk *et al.* 2013) using the BayesHammer algorithm (Nikolenko and Alekseyev 2011) and pair-end aligned with PEAR (Zhang *et al.* 2014) within PANDASeq (Masella *et al.* 2012). The quality filtered, error corrected and pair-end aligned sequences were then depreplicated, sorted by their abundance and OTU centroids picked using VSEARCH (Rognes *et al.* 2016) at the 97% level. All singleton OTUs were removed, along with all chimeric sequences using denovo chimera checking with UCHIME (Edgar *et al.* 2011). Taxonomy assignment was performed with the RDP Classifier (Wang *et al.* 2007) against the UNITE database (Koljalg *et al.* 2014).

4.3.6 Statistical Methods

Before beginning data analysis those OTUs that were not assigned at least at the phylum level (i.e. those that were only classified at the kingdom level as fungi) were removed. One sample was deemed low quality on the basis of having less than 1000 reads and was therefore excluded from the analysis. The OTUs from the three replicate sediment samples taken at the same site and at the same time were pooled together. To account for uneven sequencing depth, data were then rarified to the number of reads in the smallest pool (20,298 reads) using the vegan package in R (Jari Oksanen, F. Guillaume Blanchet *et al.* 2016). While rarefaction has been criticized for discarding valid data and reducing statistical power (McMurdie and Holmes 2014) it was shown to perform as well as, and in some cases better than other normalization techniques when low quality samples were removed before analysis (Weiss *et al.* 2015).

Principal Coordinates Analysis (PCoA), an unconstrained ordination technique, was used to visualize differences between fungal communities. To avoid overestimating

community similarity resulting from the absence of a particular OTU in multiple samples, data were standardized using a Hellinger transformation. We tested associations between the measured water and sediment chemistry variables and the PCoA axes using Pearson's product moment correlation coefficient (i.e. determined the axis loadings). Variation partitioning was conducted to determine the influence of sets of predictor variables on fungal community structure: chemical, hydrological and spatial (Table 4.1). Chemistry variables included surface water chemistry measurements and concentrations of various sediment carbon compounds (i.e. chlorophyll A, phaeopigments and total carbon); a subset of important chemistry variables to be included in the final chemistry predictor matrix for variation partitioning was determined using forward selection. To avoid selecting predictor variables that were simply descriptive of the underlying geology, the mean value for each predictor within one site and season (n = 3) was subtracted from the measured value. This retained the variability of the original dataset while setting the mean value for each predictor to zero, therefore predictors with biological significance would be less likely to be overshadowed by predictors that serve as proxies of geological differences (shown to strongly influence fungal community structure and not resulting from clustering of sites of differing geologies in space; see Figure 1). Forward selection using the ordistep function (vegan package, R) was then conducted (across all seasons) to determine the most important chemistry variables; these variables were then used in the chemistry predictor matrix (Jari Oksanen, F. Guillaume Blanchet *et al.* 2016). The hydrology predictor used was the BFIHOST value for each site. To account for spatial patterns, a Principal Components of Neighbor Matrices (PCNM) analysis was conducted, and the significant axes (here, only the first axis) was included in the spatial predictor matrix.

Table 4.1 Variables included in each variation partitioning predictor matrix

Predictor Matrix	Variable(s)
Chemical	total carbon potassium
Hydrological	BFIHOST
Spatial	PCNM axis 1

Once the key predictor variables were determined or calculated, variation partitioning was conducted for each season separately using the varpart function in vegan with Hellinger transformed data. Indictor species analysis was conducted using the package indicspecies, multipatt command multipatt with nperm=999.

4.4 Results

There were a total of 2,051,290 reads and 12,991 OTUs from our seasonal sediment samples (20,298- 124,748 reads per pooled sample), and a total of 1,317,711 reads and 10,664 OTUs in the patch-type samples (20,306-108,049 reads per pooled sample). In the clay samples, we observed huge spikes in fungal abundance (as quantified by read number; greater than 100K total reads per pooled sample) in the summer. Chalk samples also typically reach peak fungal abundance in the summer except one site with a peak in spring; the same pattern was observed in the Greensand samples. The highest overall fungal abundances were observed in clay samples in the summer, and the lowest in autumn samples from the Chalk sites. Clay sites were dominated by the yeast *Cryptococcus podzolicus*, the hyphomycete *Pseudeuotium hygrophilum*, Mortierella, and unidentified fungi in the class Sordariomycetes – the latter two also common at the sand sites along with seasonal spikes in *Rhizophydium littoreum*, a parasite of green algae. An unidentified fungus from the phylum Ascomycota by far numerically dominated at all Chalk sites across all seasons.

To visualize differences between fungal communities, PCoA was employed. Fungal communities clustered based on the underlying geology and the first two PCoA axes presented explain 31.33% of variation between these communities (Figure 4.2). The axis loadings (i.e. predictors variables with >35% correlation with a PCoA axis; Pearson's product moment correlation coefficient) were as follows: axis 1: sulphate (-0.45), calcium (-0.38), extracellular polymeric substances (extracted in 30% ethanol) (-0.38); hot-water extracted colloidal carbohydrates (-0.37), magnesium (-0.36); no predictor variable had a correlation >35% with axis 2 (Table 4.2).

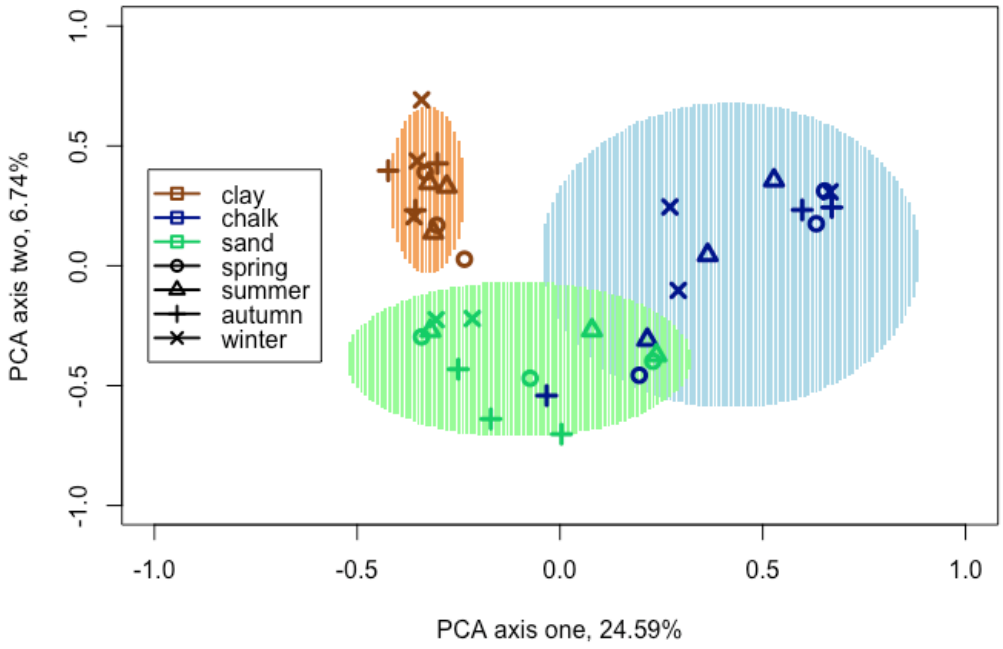

Figure 4.2 Sediment fungal communities; PCoA
Shaded areas represent mean PCoA score ± 2 standard deviations for samples within a given geology type.

Table 4.2 Water and sediment chemistry variables and PCoA axis loadings

	Chemistry parameter	Axis 1	Axis 2
surface water chemistry	nitrate	-0.232	0.162
	nitrite	-0.169	-0.064
	phosphorus	0.189	0.038
	sulphate	-0.450	0.232
	calcium	-0.376	-0.033
	magnesium	-0.359	0.080
	potassium	-0.197	0.178
	sodium	-0.116	-0.100
sediment chemistry	total carbon (TC)	0.136	0.027
	total organic carbon (TOC)	-0.034	-0.039
	chlorophyll a	-0.099	-0.017
	phaeopigment	0.327	-0.201
	colloidal carbohydrates	-0.268	0.149
	hot water extracted carbohydrates	-0.367	-0.056
	extracellular polymeric substances (30% Et-OH)	-0.379	0.338
	extracellular polymeric substances (70% Et-OH)	-0.314	-0.001

To determine the importance of the measured predictor variables, a variation partitioning analysis was conducted (Figure 4.3). Three predictor variable matrices were employed containing chemical, spatial (i.e. significant PCNM axes) and hydrological (i.e. BFIHOST values) predictors. Forward selection was conducted to determine meaningful chemical predictors to include in the predictor matrices, using season as a categorical variable. Invariably, season emerged from the analysis as a highly significant predictor ($p = 0.005$) and total carbon (TC) and potassium were the resulting chemistry variables (Table 4.1). The measured predictors explained about 20% of variation among fungal communities in spring, summer and autumn, but very little in winter. In spring and autumn, hydrology explained the highest proportion of variation and in summer hydrological and chemical predictors had about equal importance. In no season does the spatial predictor matrix provide additional explanatory power beyond that explained when hydrology and chemistry variables are included in the model.

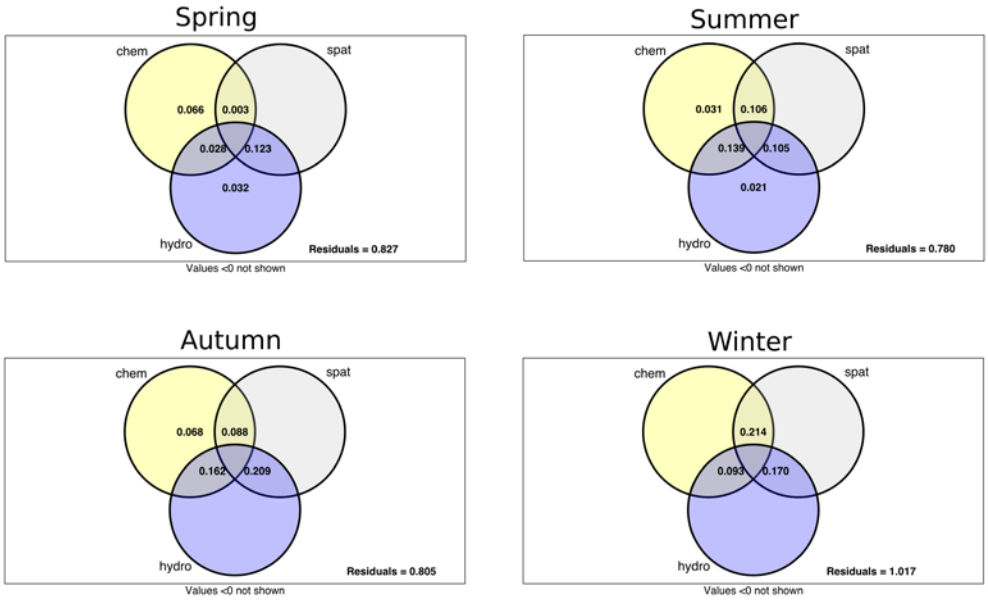

Figure 4.3. Results of the variance partitioning analysis by season

Indicator species analysis was conducted to determine which fungal OTUs were specific to particular underlying geologies. The highest number of indicator species was found in the clay samples, followed by the sand sites, with very few species specific to the Chalk sites (Table 4.3).

Table 4.3 Indicator OTUs by geology; total number of OTUs significant at p = 0.05

Geology	Chalk	Clay	Sand	Chalk & clay	Chalk & sand	Clay & sand
Total # OTUs	13	196	62	8	6	104

Half of the indicator species at the clay sites were aquatic hyphomycete species (decomposers of leaf litter); an expected outcome given the abundance of riparian trees present at these sites. Indicators specific to the sand sites were related to the surrounding terrestrial environment; these species likely originated from soil and livestock. Taxonomic information about the indicator species significant at the p = 0.005 level can be found in the Appendix, Table A4.2.

4.5 Discussion

In this study we used high-throughput sequencing techniques to investigate patterns of general fungal diversity in benthic river sediments across a sub-catchment in the Hampshire Avon. Sampling sites were located in river reaches underlain by clay, Chalk and Greensand parent materials. Distinct fungal communities inhabited the benthic sediments of the differing geologies. No previous studies have investigated the influence of geology on freshwater fungal communities using a replicated study design. Results from previous microbial ecology studies that have identified fungal communities differences among sites with differing underlying geologies/parent materials in soils (Wagai *et al.* 2011; Herold *et al.* 2014; Yarwood *et al.* 2014; Alfaro *et al.* 2017) mainly attributed this to differences in soil chemical and physical properties and indirectly to competition with plants and litter quality.

To gain insight about parameters driving the observed differences in fungal communities between sites, we conducted a variation partitioning analysis. To account for the influence of spatial structures and stochastic processes, we used significant PCNM axes as a predictor matrix in the model. We found that space did not explain any additional variation among communities that was not accounted for by hydrology and chemistry. This indicates that stochastic processes were not responsible for structuring the observed fungal communities in our sub-catchment study area. This finding contrasts with two previous studies that found spatial processes to be important for structuring riverine communities (Miura and Urabe 2015b; Liu *et al.* 2015). However, these catchment-scale studies sampled longitudinally from headwaters to estuary; thus their sampling designs may reveal the influence of hierarchal processes along the river continuum (Altermatt 2013), such as dispersion (Carrara *et al.* 2012) and carbon processing (Vannote *et al.* 1980). While few studies have examined general fungal communities in rivers, the existing evidence alongside our results indicate that the importance of spatial processes for lotic fungal community assembly may be dependent on scale (catchment vs. subcatchment) and connectivity of sampling sites.

Stochastic/spatial processes were not found to be important drivers of fungal community structure in this study, thus deterministic processes likely drove them. These include

biotic interactions (e.g. competition, facilitation) and abiotic environmental filtering. In the ordination analysis communities were found to cluster by underlying geology. Geology can influence a wide range of environmental parameters, including hydrology, benthic and surface water chemistry, and land use – and additionally at our sites both autochthonous and allochthonous vegetation differed by geology. We tested the influence of water/sediment chemistry, carbon resources and base flow on fungal community structure.

Variation partitioning analysis revealed that chemistry parameters (total carbon and K^+ concentrations were included in the model) explained additional variation that was not explained by hydrology (here, the proportion of total flow that is groundwater-derived) and space in spring, summer and autumn. Carbon and potassium are both important for fungal growth and metabolism and thus are important for biomass production. Fungal taxa produce varying types of enzymes for degrading carbon-containing compounds, and therefore would be expected to have different responses to the availability of different quantities and qualities of carbon resources. This is reflected in our data; the total carbon quantity (along with K^+ concentration) explained some differences between communities revealed by the variation partitioning analysis, and several of the most highly correlated axis loadings along PCoA axis 1 were different types of carbon compounds (i.e. community dissimilarity was correlated with carbon quality). Water and sediment chemistry explained some variation between communities in spring, summer and autumn but not winter, indicating that this may be connected to periods of warmer temperature and likely higher levels of microbial activity.

To further assess the potential influence of carbon quality, we investigated autochthonous and allochthonous carbon sources. We analyzed the fungal communities inhabiting three different patch types: unvegetated, vegetated and marginal bank sediments (two of nine sites) and riparian tree diversity (six of nine sites), which has been found to affect lotic carbon metabolism (Graham *et al.* 2017) and therefore could affect the activity and abundance of fungal taxa. Contrary to our expectation that macrophyte presence would influence fungal community structure, we did not observe separation of communities by patch type (see Appendix, Figure A4.1). Resulting from differing land management in the

different geologies, we found that riparian tree diversity was highly collinear with BFIHOST values and thus excluded it from our analysis (see Appendix, Table A4.1 and Figure A4.2). Despite differences in the quantity of autochthonous and allochthonous vegetation among our sites with differing parent material, we could not confirm that these differences were a driver in the separation in fungal communities by geology due to the limitations of our data.

Geology was also correlated with differences in hydrology: Chalk sites had high baseflow values (i.e. higher inputs of groundwater), while Greensand had intermediate and clay sites had relatively low baseflow values. The mixing of groundwater and surface water has been shown in previous studies to influence microbial diversity in the hyporheic zone (Stegen *et al.* 2016). Similar dynamics are likely also at play in the benthic zone. We hypothesized that the proportion of groundwater contributing to total stream discharge (i.e. baseflow) would influence benthic fungal community assembly. Indeed baseflow independently explained a small proportion of variation among communities in spring and summer. This could be explained by differences in water chemistry: a study conducted at six of nine of the study sites investigated here found a negative correlation between the DOC:nitrate molar ratio and BFI (Heppell *et al.* 2017). Shifts in fungal community structure have been linked to C:N ratio in soils (Lauber *et al.* 2008) and on leaf litter in streams (Kominoski *et al.* 2009). In addition to influencing surface water chemistry, upwelling groundwater also transports a unique microbial community to benthic sediments. A community coalescence (Rillig *et al.* 2015) event occurs when this groundwater community mixes with the resident benthic community. It is likely that the proportion of upwelling groundwater affects the outcome of this event: if more groundwater microorganisms are transported to benthic sediments, they have a higher chance of encountering conditions conducive to establishment.

We attempted to disentangle how hydrologic and chemical properties among the sites of differing geology may be driving the observed differences in fungal communities inhabiting sites of differing geology. However, we did not assess land use, which has been shown to influence lotic fungal community structure (Lecerf and Chauvet 2008; Miura and Urabe 2015a). There was a correlation between land-use and geology at the

Hampshire Avon sites – crop production largely takes place in the Chalk sites, whereas livestock production is predominant in the clay sites and the Greensand sites have intermediate land use. The results of the indicator species analysis indicated that land use might have played a role in shaping the benthic fungal communities. For example, two fungi from the order Onygenales, which live on keratin and are associated with mammals, were indicators of the Greensand sites. At these sites, cows were allowed access to the stream upstream of the sampling sites and intermediate BFIHOST values at the Greensand sites mean that a non-negligible proportion of discharge is attributed to overland flow/throughflow, flows that carry with them microorganisms (Crump *et al.* 2012). Many of the indicators of clay geology were aquatic hyphomycete species, classically studied lotic decomposers that break down allochthonous leaf litter, which was plentiful and diverse at these sites.

In this study we found that benthic riverine fungal communities clustered by geology and that deterministic processes were more important than stochastic processes in shaping fungal community structure at our sub-catchment study scale. Despite widespread application of high-throughput sequencing to investigate other environmental microbial communities (e.g. soil), general lotic fungal diversity remains poorly resolved. The vast majority of studies of fungal communities in rivers have focused on litter decomposers. While they perform an important ecosystem function it is likely that this is not the only role that fungi play in riverine nutrient cycling and biogeochemical cycles. Focusing on fungal communities in less-studied compartments like sediments, which are known to be hotbeds of biogeochemical activity (Battin *et al.* 2016), may uncover additional roles that fungi play in riverine systems. River systems are heavily affected by anthropogenic stressors (Dudgeon *et al.* 2006), which have been shown to affect aquatic fungal communities (Lecerf and Chauvet 2008; Cornut *et al.* 2012; Colas *et al.* 2016). It is imperative to continue to improve our understanding of the structure and function of these communities to appropriately manage river systems and ensure the maintenance of important ecosystem functions and services.

4.6 Acknowledgements

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4.7 References

Alfaro FD, Manzano M, Marquet PA, and Gaxiola A. 2017. Microbial communities in soil chronosequences with distinct parent material: the effect of soil pH and litter quality. *J Ecol* **105**: 1709–22.

Allen DJ, Darling WG, Davies J, *et al.* 2014. Groundwater conceptual models: implications for evaluating diffuse pollution mitigation measures. *Q J Eng Geol Hydrogeol* **47**: 65–80.

Altermatt F. 2013. Diversity in riverine metacommunities: A network perspective. *Aquat Ecol* **47**: 365–77.

Bärlocher F and Corkum M. 2003. Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. *Oikos* **101**: 247–52.

Bärlocher F and Graça M a. S. 2002. Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. *Freshw Biol* **47**: 1123–35.

Bärlocher F, Seena S, Wilson KP, and Dudley Williams D. 2008. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshw Biol* **53**: 368–79.

Battin TJ, Besemer K, Bengtsson MM, *et al.* 2016. The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol* **14**: 251–63.

Baudoin JM, Guérold F, Felten V, *et al.* 2008. Elevated aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf-litter breakdown. *Microb Ecol* **56**: 260–9.

Bever JD, Dickie IA, Facelli E, *et al.* 2010. Rooting theories of plant community ecology in microbial interactions. *Trends Ecol Evol* **25**: 468–78.

Bloomfield JP, Allen DJ, and Griffiths KJ. 2009. Examining geological controls on baseflow index (BFI) using regression analysis: An illustration from the Thames Basin, UK. *J Hydrol* **373**: 164–76.

Carrara F, Altermatt F, Rodriguez-Iturbe I, and Rinaldo a. 2012. Dendritic connectivity controls biodiversity patterns in experimental metacommunities. *Proc Natl Acad Sci* **109**: 5761–6.

Chauvet E, Cornut J, Sridhar KR, *et al.* 2016. Beyond the water column: aquatic hyphomycetes outside their preferred habitat. *Fungal Ecol* **19**: 112–27.

Clivot H, Cornut J, Chauvet E, *et al.* 2014. Leaf-associated fungal diversity in acidified streams: Insights from combining traditional and molecular approaches. *Environ Microbiol* **16**: 2145–56.

Colas F, Baudoin JM, Chauvet E, *et al.* 2016. Dam-associated multiple-stressor impacts on fungal biomass and richness reveal the initial signs of ecosystem functioning impairment. *Ecol Indic* **60**: 1077–90.

Cornut J, Chauvet E, Mermillod-Blondin F, *et al.* 2014. Aquatic hyphomycete species are screened by the hyporheic zone of woodland streams. *Appl Environ Microbiol* **80**: 1949–60.

Cornut J, Clivot H, Chauvet E, *et al.* 2012. Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams. *Water Res* **46**: 6430–44.

Crump BC, Amaral-Zettler L a, and Kling GW. 2012. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME J* **6**: 1629–39.

Das M, Royer T V, and Leff LG. 2007. Diversity of Fungi, Bacteria, and Actinomycetes on Leaves Decomposing in a Stream. *Appl Environ Microbiol* **73**: 756–67.

Das M, Royer T V., and Leff LG. 2008. Fungal communities on decaying leaves in streams: A comparison of two leaf species. *Mycol Prog* **7**: 267–75.

Duarte S, Bärlocher F, Trabulo J, *et al.* 2015. Stream-dwelling fungal decomposer communities along a gradient of eutrophication unraveled by 454 pyrosequencing. *Fungal Divers* **70**: 127–48.

Duarte S, Fernandes I, Nogueira MJ, *et al.* 2013. Temperature alters interspecific relationships among aquatic fungi. *Fungal Ecol* **6**: 187–91.

Duarte S, Pascoal C, Alves A, *et al.* 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. *Freshw Biol* **53**: 91–101.

Duarte S, Pascoal C, and Cássio F. 2016. Biogeography of aquatic hyphomycetes: Current knowledge and future perspectives. *Fungal Ecol* **19**: 169–81.

Dudgeon D, Arthington AH, Gessner MO, *et al.* 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol Rev* **81**: 163.

Edgar RC, Haas BJ, Clemente JC, *et al.* 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**: 2194–200.

Fernandes I, Pascoal C, Guimarães H, *et al.* 2012. Higher temperature reduces the effects of litter quality on decomposition by aquatic fungi. *Freshw Biol* **57**: 2306–17.

Ferreira V and Chauvet E. 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Glob Chang Biol* **17**: 551–64.

Graham EB, Tfaily M, Crump AR, *et al.* 2017. Carbon inputs from riparian vegetation limit oxidation of physically-bound organic carbon via biochemical and thermodynamic processes. *bioRxiv*.

Gulis and K. Suberkropp V. 2003. Leaf litter decompositioin and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshw Biol* **48**: 123–34.

Hanlon ARM, Bellinger B, Haynes K, *et al.* 2006. Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersio-immersion period. *Limnol Oceanogr* **51**: 79–93.

Heppell CM, Binley A, Trimmer M, *et al.* 2017. Hydrological controls on DOC:nitrate resource stoichiometry in a lowland, agricultural catchment, southern UK. *Hydrol Earth Syst Sci Discuss*: 1–42.

Herold N, Schöning I, Gutknecht J, *et al.* 2014. Soil property and management effects on grassland microbial communities across a latitudinal gradient in Germany. *Appl Soil Ecol* **73**: 41–50.

Hoellein TJ, Tank JL, Kelly JJ, and Rosi-Marshall EJ. 2010. Seasonal variation in nutrient limitation of microbial biofilms colonizing organic and inorganic substrata in streams. *Hydrobiologia* **649**: 331–45.

Ihrmark K, Bödeker ITM, Cruz-Martinez K, *et al.* 2012. New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* **82**: 666–77.

Jari Oksanen, F. Guillaume Blanchet M, Friendly, Roeland Kindt, Pierre Legendre D, McGlinn, Peter R. Minchin, R. B. O'Hara GL, *et al.* 2016. vegan: Community Ecology Package.

Joshi N and Fass J. 2011. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files.

Koljalg U, Nilsson RH, Abarenkov K, *et al.* 2014. Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* **22**: 5271–7.

Kominoski JS, Hoellein TJ, Kelly JJ, and Pringle CM. 2009. Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species? *Oikos* **118**: 457–63.

Krauss G-J, Solé M, Krauss G, *et al.* 2011. Fungi in freshwaters: ecology, physiology and biochemical potential. *FEMS Microbiol Rev* **35**: 620–51.

Lauber CL, Strickland MS, Bradford MA, and Fierer N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* **40**: 2407–15.

Lecerf A and Chauvet E. 2008. Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshw Biol* **53**: 1658–72.

Leff JW, Jones SE, Prober SM, *et al.* 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc Natl Acad Sci* **112**: 10967–72.

Liu J, Wang J, Gao G, *et al.* 2015. Distribution and diversity of fungi in freshwater sediments on a river catchment scale. *Front Microbiol* **6**: 1–12.

Maeda K, Spor A, Edel-Hermann V, *et al.* 2015. N2O production, a widespread trait in fungi. *Sci Rep* **5**: 9697.

Masella AP, Bartram AK, Truszkowski JM, *et al.* 2012. PANDAseq: Paired-end assembler for illumina sequences. *BMC Bioinformatics* **13**: 1–7.

McMurdie PJ and Holmes S. 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Comput Biol* **10**.

Miura A and Urabe J. 2015a. Riparian land cover and land use effects on riverine epilithic fungal communities. *Ecol Res* **30**: 1047–55.

Miura A and Urabe J. 2015b. Spatial and seasonal changes in species diversity of epilithic fungi along environmental gradients of a river. *Freshw Biol* **60**: 673–85.

Nikolcheva LG and Barlocher F. 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environ Microbiol* **7**: 270–80.

Nikolenko SI and Alekseyev M. 2011. {BAYESHAMMER}: Bayesian subclustering for error correction in single cell sequencing. **14**: 1–11.

Nurk S, Bankevich A, Antipov D, *et al.* 2013. Assembling genomes and mini-metagenomes from highly chimeric reads. In: Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics). Berlin: Springer Verlag.

Pascoal C and Cássio F. 2004. Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River. *Appl Environ Microbiol* **70**: 5266–73.

Rillig MC, Antonovics J, Caruso T, *et al.* 2015. Interchange of entire communities: microbial

community coalescence. *Trends Ecol Evol* **30**: 470–6.
Rillig MC and Mummey DL. 2006. Mycorrhizas and soil structure. *New Phytol* **171**: 41–53.
Rognes T, Flouri T, Nichols B, *et al.* 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ Prepr* **4**: e2409v1.
Rolls RJ, Heino J, Ryder DS, *et al.* 2017. Scaling biodiversity responses to hydrological regimes. *Biol Rev*.
Rubin MA and Leff LG. 2007. Nutrients and other abiotic factors affecting bacterial communities in an Ohio River (USA). *Microb Ecol* **54**: 374–83.
Shearer C, Descals E, Kohlmeyer B, *et al.* 2007. Fungal biodiversity in aquatic habitats. *Biodivers Conserv* **16**: 49–67.
Stal LJ, Gemerden H van, and Krumbein WE. 1984. The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. *J Microbiol Methods* **2**: 295–306.
Stegen JC, Fredrickson JK, Wilkins MJ, *et al.* 2016. Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. *Nat Commun* **7**: 11237.
Suberkropp K and Chauvet E. 1995. Regulation of Leaf Breakdown by Fungi in Streams: Influences of Water Chemistry. *Ecology* **76**: 1433–45.
Thompson LR, Sanders JG, McDonald D, *et al.* 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**: 457–63.
Tornwall B, Sokol E, Skelton J, and Brown B. 2015. Trends in Stream Biodiversity Research since the River Continuum Concept. *Diversity* **7**: 16–35.
Vannote RL, Minshall GW, Cummins KW, *et al.* 1980. The River Continuum Concept. *Can J Fish Aquat Sci* **37**: 130–7.
Veresoglou SD, Chen B, and Rillig MC. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol Biochem* **46**: 53–62.
Wagai R, Kitayama K, Satomura T, *et al.* 2011. Interactive influences of climate and parent material on soil microbial community structure in Bornean tropical forest ecosystems. *Ecol Res* **26**: 627–36.
Wang Q, Garrity GM, Tiedje JM, and Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**: 5261–7.
Weiss SJ, Xu Z, Amir A, *et al.* 2015. Effects of library size variance, sparsity, and compositionality on the analysis of microbiome data. *PeerJ Prepr* **3**: e1408.
Yarwood S, Wick A, Williams M, and Daniels WL. 2014. Parent Material and Vegetation Influence Soil Microbial Community Structure Following 30-Years of Rock Weathering and Pedogenesis. *Microb Ecol* **69**: 383–94.
Yu S-X, Pang Y-L, Wang Y-C, *et al.* 2017. Spatial variation of microbial communities in sediments along the environmental gradients from Xiaoqing River to Laizhou Bay. *Mar Pollut Bull* **120**: 90–8.
Zeglin LH. 2015. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Front Microbiol* **6**: 1–15.
Zhang J, Kobert K, Flouri T, and Stamatakis A. 2014. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–20.

5. COLLEMBOLANS IMPACT SOIL NITROUS OXIDE PRODUCTION

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5.1 Abstract

Soils represent a major source of the greenhouse gas nitrous oxide, produced in part as an end product of denitrification. While the physical and chemical controls on denitrification have been well studied, biotic drivers have not been well characterized. The effect of Collembola, ubiquitous and widespread soil microarthropods, has only been investigated in a few studies and under artificial conditions. Here we investigate the influence of natural field densities of two species of collembolans on potential denitrification in a plant-soil system. We found an increased proportion of nitrous oxide as an end product of denitrification in both collembolan treatments. Because collembolans are sensitive to environmental change, these findings could have implications for denitrification in impacted environments.

5.2 Introduction

Denitrification is an anaerobic ecosystem process that involves the stepwise reduction of oxidized inorganic nitrogen species and terminates with the production of the gaseous end-products dinitrogen (N_2) and nitrous oxide (N_2O), a powerful greenhouse gas. This process is carried out by a phylogenetically diverse group of microorganisms, including bacteria, archaea and fungi (Philippot *et al.* 2007; Maeda *et al.* 2015). In riparian zones buffering agricultural land, denitrification may serve to lessen the load of nitrate from agricultural runoff entering water bodies (Boz *et al.* 2013). However, N_2O, a product of denitrification, is a greenhouse gas with a 120 year atmospheric lifetime (Braker and Conrad 2011). Denitrification in riparian zones is an important component of terrestrial nitrous oxide fluxes (Bouwman *et al.* 2013).

The physical and chemical controls on denitrification are relatively well characterized. Oxygen, nitrate and carbon concentrations, soil pH and temperature affect the instantaneous rates of denitrification, while denitrifier community composition is driven

by both abiotic and biotic factors, such as predation (Wallenstein *et al.* 2006). A recent review of the drivers and controls of denitrification concludes that a deeper understanding of microbial composition and diversity is required to better characterize terrestrial nitrous oxide fluxes (Butterbach-Bahl *et al.* 2013).

There is some evidence that soil biota affect denitrifier community composition and activity. Increased (Marhan *et al.* 2015) and decreased (Kuiper *et al.* 2013) N_2O emissions have been observed in the presence of earthworms. Soil fungi have been shown to affect the distribution of bacterial denitrifier taxa (Burke *et al.* 2012) and arbuscular mycorrhizal fungi have been reported to reduce soil N_2O emissions (Bender *et al.* 2014). Soil invertebrate grazers such as Collembola could exert a direct influence on denitrifiers and denitrification by releasing nutrients (Teuben and Verhoef 1992; Bardgett and Chan 1999) and indirectly through altering soil fungal community composition and activity (Crowther *et al.* 2012).

Collembolans are ubiquitous and globally-distributed soil animals, found in ecosystems ranging from the tropics to the arctic, and are among the most abundant terrestrial arthropods (Hopkin 1997). Despite their potential to directly and indirectly affect denitrification, only two studies have investigated collembolan effects on denitrification. In one study comparing the influence of various soil fauna on denitrification, there was a trend of increased N_2O emissions in the collembolan treatment but they were not signficantly different than the control (Kuiper *et al.* 2013); another study observed no difference in N_2O emissions but their data indicated a shift from a fungal to a bacterial denitrification pathway (Schorpp *et al.* 2016). Both of these studies were conducted using unrealistically high densities of collembolans compared to those in the field and this may have induced an unnatural response in the soil fungi: at high densities fungal growth and activity is reduced in response to invertebrate grazing, while at lower densities growth and activity can be stimulated (Crowther *et al.* 2012). A third study reports a significant increase in N_2O emissions in the presence of collembolans (Wu *et al.* 2015). Thus the influence of collembolans on denitrification under natural conditions is not well characterized and existing results are contradictory.

We performed a pot experiment to investigate the effect of two species of collembolans, *Folsomia candida* and *Proisotoma minuta* on denitrification. We attempted to mimic natural conditions by using a plant-soil system and adding each collembolan species at a density typical for that species in the field. We hypothesized that there would be higher total potential denitrification rates in the collembolan treatment resulting from stimulation of the denitrifier community via carbon (Johnson *et al.* 2005) and nutrient (Teuben and Verhoef 1992) release (Hypothesis 1). Collembola have been reported to increase soil aggregation (Siddiky *et al.* 2012a, b), particularly of large macroaggregates thus, we also hypothesized that there would be higher potential denitrification in the collembolan treatments resulting from an increased availability of anaerobic microsites and thus higher activity of these anaerobic organisms (e.g. Ebrahimi and Or 2016; Hypothesis 2). Finally, we expected that there would be differences in N_2O emissions, likely resulting from collembolan-induced changes in microbial community composition (Hypothesis 3).

5.3 Materials and Methods

5.3.1 Climate chamber experiment

Soil was collected from an Albic Luvisol at an experimental site of the Freie Universität. This soil is sandy in texture (70% sand, 21% silt, 9% clay) with 0.3 mg/100g $CaCl_2$-extractable nitrate, 4.6 mg/100g P, and a pH of 5.9 (soil analysis conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany). Soil was sieved to 4mm and mixed 3:1 with sand by weight to reduce soil fertility and encourage the establishment of mycorrhizal fungi. The soil was then sterilized via two rounds of steam sterilization for 4 hours at 90°C. The experimental system consisted of pots containing cylindrical mesh compartments. The compartments were fabricated from coarse clear plastic mesh (2mm, Bauhaus, Germany) surrounded by a finer 38µm mesh (SEFAR GmbH, Edling, Germany) and sealed with hot glue and duct tape. This mesh size is sufficient for fungal hyphae to traverse but excludes the crossing of collembolans and plant roots.

Five-hundred grams of steam-sterilized soil were distributed into each mesh compartment, and sufficient soil to bring the pots + compartments to 1800g was added

such that that the height of soil inside and outside of the compartments was level. The mesh compartment was seated in the pot in such a way that a ~4cm lip extended above the soil surface to prevent collembolans from exiting the compartment. A separate portion of the soil was stored at 4°C following collection from the field and used to prepare a microbial filtrate containing the native soil bacteria and fungal hyphae but excluding non-microbial soil organisms and mycorrhizal fungal spores. To prepare the microbial filtrate, soil was mixed with 0.9% NaCl solution (100g/1L), shaken vigorously by hand for 1 minute, sieved through a 20µm sieve and stored at 4°C until use. Microbial filtrate (125mL) was added to each experimental unit inside and outside of the mesh compartments. Experimental units were left in the climate chamber for a 2 week incubation period to allow the re-equilibration of the microbial community (Shaw *et al.* 1999). Climate chamber conditions included a 16h/8h light/dark cycle with an ambient temperature of 20°C during the light period and 15°C during the dark period. Pots were maintained at 60% water holding capacity (WHC) via thrice weekly watering and their position randomized weekly for the course of the experiment.

Plantago lanceolata seeds (Appels Wilde Samen, Darmstadt, Germany) were surface-sterilized, first with 70% ethanol for 2 min. and then with a solution of 5% bleach and 0.05% SDS for 5 minutes. Seeds were then rinsed five times with autoclaved deionized water. Following the soil incubation period, three seeds were added to each pot outside of the mesh compartments. Plants were used in our experimental units as a host for arbuscular mycorrhizal (AM) fungi and to more realistically imitate soil water dynamics in the soil system. Seeds were planted at a 1cm depth after 30mg of dry inoculum (Symplanta GmbH & Co. KG, Oldenburg, Germany) of the AM fungus *Rhizophagus irregularis* was mixed into the top 3cm of soil outside of the mesh compartment. Autoclave-sterilized inoculum was added to the non-mycorrhizal treatments (n = 30). To prevent drying of seedlings resulting from air circulation in the climate chamber, pots were covered with film plastic and seedlings were sprayed twice daily with deionized water for 2 weeks. Seedlings were thinned so that each pot contained one plant. After a 5-week period to allow for plant establishment and mycorrhizal growth, collembolans were added into the mesh compartments. Mimicking natural field densities of each collembolan species (Maass, unpub. data) we applied the following treatments: 12

Folsomia candida individuals (FC), 30 *Proisotoma minuta* individuals (PM) or no collembolans (NC) were added to 10 pots each with and without *R. irregularis* inoculum (60 total experimental units).

5.3.2 Measurement of biotic and soil parameters

Ten weeks after the addition of collembolans, experimental units were destructively harvested. Plants were cut at the soil surface, fresh weights of aboveground biomass measured and then placed into a 40°C drying oven. Plant dry biomasses were taken after samples reached a constant weight. Soil was harvested from the mesh compartment of the experimental units only. A 125g portion of soil was transferred to mesh-bottom containers and placed into a modified MacFadyen apparatus for collembolan extraction. The temperature was increased in 2°C increments from 25°C to 50°C over a period of two weeks after which collembolans were counted and survival rates (# added/#extracted) were calculated. The remaining soil from the collembolan extraction was dried at 60°C and used to measure the percentage of water-stable soil aggregates (%WSA) using a wet sieving apparatus (Eijkelkamp, The Netherlands) and a 5 minute sieving time using the methods of (Kemper and Rosenau 1986). Soil nitrate, ammonium and organic carbon were extracted using concentrated KCl. Briefly, a 2M KCl solution was mixed with soil in a 1:5 ratio (g soil to mL KCl), shaken at 175rpm for 1hr, centrifuged at 3400rpm for 10 minutes and supernatant filtered through a 0.7µm glass microfiber filter (Whatman, Maidstone, UK). Nitrate and ammonium were measured using segmented flow analysis (Skalar San^{++}, Breda, the Netherlands) and organic carbon was determined using a TOC analyzer. AM fungal hyphae were extracted from a 4g soil sample following (Jakobsen *et al.* 1992), stained with Trypan Blue and length was measured via microscopic quantification of hyphae at 200X (Rillig *et al.* 1999). AM hyphae were quantified in about 1/3 of the samples and no significant differences in AM hyphal length were observed between AM and non-AM treatments. This was verified by lack of mycorrhizal structures in several ink-stained root samples (prepared and quantified using methods described in (Vierheilig *et al.* 1998; Rillig *et al.* 1999)). Due to lack of evidence that the mycorrhizal treatment effectively developed, we removed all AM samples (n=30) from analysis.

5.3.3 Denitrification enzyme activity measurement

Soil was stored at 4°C for denitrification enzyme activity (DEA) measurements, which were made within 7 days of the harvest. DEA is a measurement of the maximum potential denitrification activity of a community under ideal conditions (i.e. non-limiting substrate availability and anaerobic conditions). We measured DEA in two replicate 25g soil samples from each mesh compartment, with and without acetylene (4 total measurements per compartment). Soil was placed in a 125mL screw-top plastic media bottle with a lid fitted with a (rubber) septum along with 15mL of a 1mM glucose and 2.37mM KNO_3 solution (Philippot *et al.* 2013), to provide carbon and nitrate, and chloramphenicol (0.7mM), to prevent the synthesis of new enzymes during the course of the assay. To produce an anaerobic environment, the headspace of the bottles was flushed for 5m with N_2 gas and then brought to ambient pressure. Acetylene gas was produced by adding deionized water to calcium carbide and 13mL of acetylene was added to half the bottles. The addition of acetylene blocks the final step in the denitrification pathway ($N_2O \rightarrow N_2$), thus allowing the quantification of total denitrification through measuring N_2O. Bottles were then incubated at 25°C protected from light and 4mL gas samples were collected at 45, 90, 150 and 210min and transferred to 5.9 mL Exetainer® vials (Labco, Lampeter, UK) previously flushed with N_2. Samples were diluted with 8mL of N_2 gas immediately after sample collection. Nitrous oxide concentrations were measured using an Agilent 7890 gas chromatograph equipped with an electron capture detector (Agilent Technologies Inc., Santa Clara, CA, USA). The N_2O concentrations were corrected to account for the dilution and then regressed against elapsed time (in hours) over which samples were collected; any sample whose rate regression did not meet the pre-established quality criterion of an R^2 above 0.75 (i.e. linear production rate) was excluded from further analysis. Rate data were then corrected with the appropriate Bunsen coefficient and using soil moisture content and headspace volume to account for dissolved N_2O. Total denitrification rates and N2O production rates are reported here as μg N_2O-N per hour per gram dry weight soil.

5.3.4 Statistical methods

All statistical analyses were performed using R version 3.3.1 (R Core Team 2016). To verify that treatment differences resulted from the differing collembolan treatments and not plant effects, we assessed plant biomass across treatment groups using univariate analysis of variance (ANOVA). We then used ANOVA to assess the effect of collembolan treatment on four response variables: %WSA, total denitrification (i.e. $N_2O + N_2$), N_2O production, and the $N_2O/(N_2O + N_2)$ log response ratio. The Shapiro-Wilk normality test was used to assess normality of residuals and Bartlett's test was used to test homogeneity of variance of the data. We tested associations between the measured predictor variables (i.e. soil nitrate, ammonium total inorganic nitrogen (nitrate + ammonium), organic carbon concentrations, collembolan survival rates and %WSA) and the denitrification response variables using Pearson's product moment correlation coefficient. Finally, we ran a linear model correlating the $N_2O/(N_2O + N_2)$ log response ratio with significant predictor variables. As the assumption of homogeneity of variance was only marginally fulfilled and sample size was small, the linear model was fitted using generalized least squares (GLS) with a variance correction structure using the nlme package in R (Pinheiro *et al.* 2017), and then using Akaike's Information Criterion to select the best-fitting model.

5.4 Results

5.4.1 Confirmation of intended experimental design

We found that dry plant biomass did not significantly differ between treatments ($p = 0.27$) therefore we assume that the observed differences between treatment groups originated from the applied treatments and not from differential effects resulting from plant nutrient or water uptake. As described in section 5.3.2, the development of AM hyphae was not observed in the soil nor AM structures in plant roots, thus this treatment failed to develop (30/60 pots). The remainder of this manuscript details the results from the collembolan treatments only. In the non-mycorrhizal treatments, plants did not survive to the end of the experiment in two of the pots, thus from the original 60 experimental units, we report data from 28. At the end of the experiment, survival rates

for *F. candida* were 93 ± 26% and were 280 ± 78% for *P. minuta* (mean ± S.E.), where greater than 100% survival indicates reproduction over the course of the experiment. No collembolans were present in the controls. These data verify the successful application of the collembolan treatment.

5.4.2 Effects of collembolan treatments on soil structure and denitrification

Contrary to our expectation, we did not observe differences between collembolan treatment groups with regard to soil aggregation ($p = 0.16$; Figure 5.1).

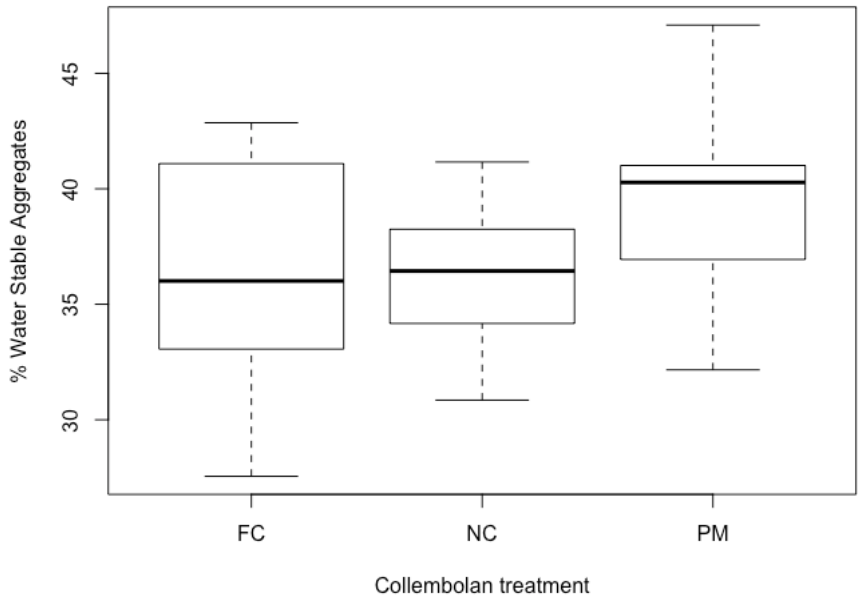

Figure 5.1 Boxplot displaying differences in %WSA between treatments
Abbreviations: FC = *F. candida*, NC = no collembolan control, PM = *P. minuta*

There were no significant differences among treatment groups in total denitrification rate or nitrous oxide production rate; however, there was a significant difference between groups in the log response ratio of total denitification rate (i.e. $N_2O + N_2$) to nitrous oxide production rate (i.e. N_2O), where we observed a higher fractional N_2O production rate in the collembolan treatments compared to the control (Table 5.1). Soil nitrate concentration was the only predictor variable that significantly correlated with the log response ratio (p

= 0.012); soil ammonium and organic carbon concentrations, collembolan survival rate and percent water stable aggregates were not significant.

Table 5.1 Differences in nine predictor and response parameters between collembolan treatment groups, mean ± standard error. Abbreviations: OC = organic carbon, WSA = water-stable aggregate, DW = dry weight soil.

Parameters	Control	*F. candida*	*P. minuta*
Collembolan survival rate	0 ± 0	0.93 ± 0.26	2.80 ± 0.78
NO_3-N (mg/kg_{DW})	4.43 ± 1.44	3.06 ± 1.39	2.05 ± 0.64
NH_4-N (mg/kg_{DW})	1.90 ± 0.14	2.01 ± 0.12	2.00 ± 0.16
OC (mg/kg_{DW})	102.43 ± 6.00	94.60 ± 9.21	112.88 ± 10.27
WSA (%)	36.36 ± 1.10	35.98 ± 1.77	39.50 ± 1.26
shoot biomass (g)	4.60 ± 0.49	4.38 ± 0.36	5.32 ± 0.42
$N_2O + N_2$ (µg N_2O-N/hr/g_{DW})	28.77 ± 2.03	25.74 ± 3.99	27.45 ± 4.07
N_2O only (µg N_2O-N/hr/g_{DW})	3.70 ± 0.65	4.50 ± 0.58	4.18 ± 0.60
N_2O (% of total denitrification)	10.5 ± 2.1	22.1 ± 3.5	17.7 ± 1.9

To further explore the relationship between collembolan treatment, soil nitrate concentration and the ratio of N_2O production to total denitrification, we ran a linear model (fitted with GLS) correlating the $N_2O/(N_2O + N_2)$ log response ratio with nitrate and collembolan treatment (Table 2). The treatment, nitrate concentration and their interaction were all significant. The relationship between the response ratio and soil nitrate concentration was unique within each treatment group (Figure 5.2). There is a negative relationship between these variables in the *F. candida* treatment and control, whereas there is a neutral-to-slightly positive relationship in the *P. minuta* treatment.

Table 5.2 ANOVA results from GLS-fitted linear model; response variable: $N_2O/(N_2O + N_2)$ log response ratio

	DF	F-value	p-value
Collembolan treatment	2	4.9528	0.0236
Soil nitrate (mg/kg_{DW})	1	4.3800	0.0551
Interaction	2	4.0758	0.0403

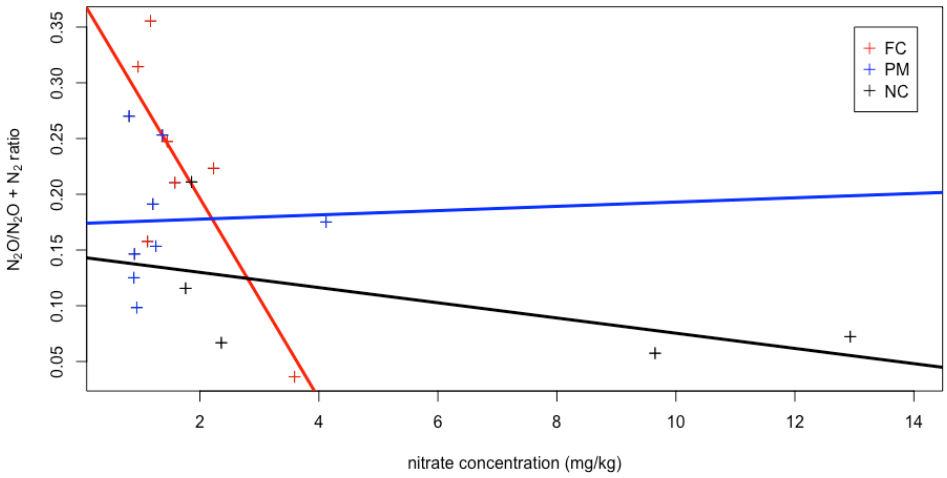

Figure 5.2 Interaction between N2O/(N2O + N2) response ratio and nitrate within collembolan treatments
The differences in slopes across the three treatments are suggestive of a significant interaction between collembolan treatment and NO_3^- availability in soil. Abbreviations: FC = *F. candida*, PM = *P. minuta*, NC = no collembolan control.

5.5 Discussion and Conclusions

This study investigated the effects of two species of collembola, *F. candida* and *P. minuta*, on soil aggregation and potential denitrification in a plant-soil system. We hypothesized that the presence of collembola would increase soil aggregate formation and alter soil denitrification compared to the collembola-free control. Contrary to our expectation and unlike earlier studies (Siddiky *et al.* 2012a, b), we did not observe collembolan effects on soil aggregation. An earlier study reported that the presence of *P. minuta* increased soil aggregation, particularly large macro-aggregate (2-4mm) formation (Siddiky *et al.* 2012b). While we did observe a slight trend towards increased water stable aggregates in the *P. minuta* treatment, it was non-significant. It is possible that differences in soil preparation (sieving to 4mm vs. 10mm), interactions with plant roots (excluded vs. allowed) and collembolan density (60 vs. 27 individuals per kg soil) could explain the differences between the results of the two studies. The latter point is likely to have influenced the outcome. AM (Rillig and Mummey 2006) and non-AM (Tisdall and Oades 1982) fungi contribute to soil aggregation, for example, through the production of secretions (e.g. proteins) and physical enmeshment; however, Collembola feed on

(preferentially non-AM) fungal hyphae (Seastedt 1984). At low densities, collembolan grazing on fungal hyphae can induce compensatory growth leading to increased fungal density (Leonard and Anderson 1982; Crowther *et al.* 2012) and therefore an increased occurrence of these aggregate-promoting processes. While we did not use particularly high densities of collembolans in this study, we may have added an amount sufficient to dampen the stimulatory growth and activity effects of grazing, reducing fungal-moderated effects on soil aggregation.

While our hypothesis regarding collembolan effects on soil aggregation was not supported by our data, we did observe differences in denitrification between our treatment groups. Specifically, we observed an increase in the ratio of nitrous oxide production to total denitrification in the collembolan treatments. There was a slight but non-significant trend in reduction of total denitrification coupled with a slight but non-significant trend in increased N_2O production in the collembolan treatments. This resulted in a significant difference in the percentage of N_2O (i.e. the response ratio) between the collembolan treatments and the control. This result could be explained by shifts in the microbial community in the presence of collembolans. A previous study investigating the influence of collembolans on denitrifier community structure reported a shift in denitrification from the fungal to the bacterial pathway when collembola were present (Schorpp *et al.* 2016). Denitrification is a multi-step pathway and the enzymes responsible for the successive reduction of nitrogen species are distributed throughout different taxa in the community (Philippot 2002). Shifts in the community composition could lead to shifts in the abundance of denitrification enzymes and thereby total denitrification activity and ratio of end products (Cavigelli and Robertson 2001; Philippot *et al.* 2013).

Changes in denitrification activity in the presence of collembolans could also be explained by shifts in substrate availability. Organic carbon and nitrate are the substrates necessary for denitrification. We observed a reduction in the soil nitrate availability in the collembolan treatments compared to the control. This may have resulted from stimulation of microbial activity by collembolans (Lussenhop 1992) and therefore increased nitrate uptake. Increased competition for nitrate with other microbial functional groups could

have resulted in a decrease in denitrifier abundance in the collembolan treatments, and therefore a decrease in overall denitrification potential. We observed a significant interaction of collembolan treatment and soil nitrate concentration on N_2O:(N_2O + N_2) ratio (Table 5.2, Figure 5.2). There was a sharp decrease in the N_2O:(N_2O + N_2) ratio with increasing nitrate concentration in the *F. candida* treatment, whereas there was lower variability and no strong relationship with nitrate concentrations in the *P. minuta* treatment. The difference between these two treatments highlights differential effects of the two species on denitrification. Though collembolans have been hypothesized to directly influence denitrification (e.g. through production of nitrate enriched fecal pellets; Lussenhop 1992) we did not find elevated nitrate levels in our collembolan treatments compared to the controls. Thus treatment differences are likely attributable to shifts in the soil microbial community.

In this study we found two species of soil collembolans to increase the proportion of N_2O produced as an end product of denitrification at natural field densities. There were differential effects of the significant species interaction with soil nitrate concentrations as it related to the N_2O:(N_2+N_2O) ratio. These data indicate that these ubiquitous soil organisms influence the denitrifier community and likely do so in a species-specific manner. This may have implications for ecosystems under anthropogenic pressure: collembolans are sensitive to environmental changes (Hopkin 1997) and shifts in their abundance and community composition in affected ecosystems may modulate denitrification in these environments. This could be particularly important in riparian zones, which are both hotspots of denitrification activity and highly threatened by anthropogenic activity.

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5.7 References

Bardgett RD and Chan KF. 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biol Biochem* **31**: 1007–14.

Bender SF, Plantenga F, Neftel A, *et al.* 2014. Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. *ISME J* **8**: 1336–45.

Bouwman AF, Beusen AHW, Griffioen J, *et al.* 2013. Global trends and uncertainties in terrestrial denitrification and N2O emissions. *Philos Trans R Soc B Biol Sci* **368**: 20130112–20130112.

Boz B, Mizanur Rahman M, Bottegal M, *et al.* 2013. Vegetation, soil and hydrology management influence denitrification activity and the composition of nirK-type denitrifier communities in a newly afforested riparian buffer. *N Biotechnol* **30**: 675–84.

Braker G and Conrad R. 2011. Diversity, structure, and size of N2O-producing microbial communities in soil—what matters for their functioning? In: Advanced in Applied Microbiology, Volume 75.

Burke DJ, Smemo K a., López-Gutiérrez JC, and DeForest JL. 2012. Soil fungi influence the distribution of microbial functional groups that mediate forest greenhouse gas emissions. *Soil Biol Biochem* **53**: 112–9.

Butterbach-Bahl K, Baggs EM, Dannenmann M, *et al.* 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc Lond B Biol Sci* **368**: 20130122.

Cavigelli MA and Robertson GP. 2001. Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biol Biochem* **33**: 297–310.

Crowther T, Boddy L, and Jones TH. 2012. Functional and ecological consequences of saprotrophic fungus-grazer interactions. **6**: 1992–2001.

Ebrahimi A and Or D. 2016. Microbial community dynamics in soil aggregates shape biogeochemical gas fluxes from soil profiles - upscaling an aggregate biophysical model. *Glob Chang Biol* **22**: 3141–56.

Hopkin SP. 1997. Biology of the Springtails (Insecta: Collembola). Oxford: Oxford University Press.

Jakobsen I, Abbott LK, and Robson a. D. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with Trifolium subterraneum L. *New Phytol* **120**: 371–380.

Johnson D, Krsek M, Wellington EMH, *et al.* 2005. Soil invertebrates disrupt carbon flow through fungal networks. *Science* **309**: 1047.

Kemper WD and Rosenau RC. 1986. Aggregate Stability and Size Distributlon. *Methods Soil Anal Part 1 - Phys Mineral Methods* **9**: 425–42.

Kuiper I, Deyn GB de, Thakur MP, and Groenigen JW Van. 2013. Soil invertebrate fauna affect N2O emissions from soil. *Glob Chang Biol* **19**: 2814–25.

Leonard PIMA and Anderson JM. 1982. Effect of collembolan grazing upon nitrogen and cation leaching from decomposing leaf litter. *Soil Biol Biochem* **14**: 601–5.

Lussenhop J. 1992. Mechanisms of Microarthropod-Microbial Interactions in Soil. *Adv Ecol Res* **23**: 1–33.

Maeda K, Spor A, Edel-Hermann V, *et al.* 2015. N2O production, a widespread trait in fungi. *Sci*

Rep **5**: 9697.
Marhan S, Auber J, and Poll C. 2015. Additive effects of earthworms, nitrogen-rich litter and elevated soil temperature on N2O emission and nitrate leaching from an arable soil. *Appl Soil Ecol* **86**: 55–61.
Philippot L. 2002. Denitrifying genes in bacterial and Archaeal genomes. *Biochim Biophys Acta - Gene Struct Expr* **1577**: 355–76.
Philippot L, Hallin S, and Schloter M. 2007. Ecology of Denitrifying Prokaryotes in Agricultural Soil. *Adv Agron* **96**: 249–305.
Philippot L, Spor A, Hénault C, *et al.* 2013. Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* **7**: 1609–19.
Pinheiro J, Bates D, DebRoy S, *et al.* 2017. nlme: Linear and Nonlinear Mixed Effects Models.
R Core Team. 2016. R: A Language and Environment for Statistical Computing (H Fehske, R Schneider, and A Weiße, Eds). *R Found Stat Comput*.
Rillig MC, Field CB, and Allen MF. 1999. Soil biota responses to long-term atmospheric CO2 enrichment in two California annual grasslands. *Oecologia* **119**: 572–7.
Rillig MC and Mummey DL. 2006. Mycorrhizas and soil structure. *New Phytol* **171**: 41–53.
Schorpp Q, Riggers C, Lewicka-Szczebak D, *et al.* 2016. Influence of *Lumbricus terrestris* and *Folsomia candida* on N_2O formation pathways in two different soils - with particular focus on N_2 emissions. *Rapid Commun Mass Spectrom* **49**: 2301–14.
Seastedt T. 1984. The Role of Microarthropods in Decomposition and Mineralization Processes. *Annu Rev Entomol* **29**: 25–46.
Shaw LJ, Beaton Y, Glover L a., *et al.* 1999. Re-inoculation of autoclaved soil as a non-sterile treatment for xenobiotic sorption and biodegradation studies. *Appl Soil Ecol* **11**: 217–26.
Siddiky MRK, Kohler J, Cosme M, and Rillig MC. 2012a. Soil biota effects on soil structure: Interactions between arbuscular mycorrhizal fungal mycelium and collembola. *Soil Biol Biochem* **50**: 33–9.
Siddiky MRK, Schaller J, Caruso T, and Rillig MC. 2012b. Arbuscular mycorrhizal fungi and collembola non-additively increase soil aggregation. *Soil Biol Biochem* **47**: 93–9.
Teuben A and Verhoef HA. 1992. Direct contribution by soil arthropods to nutrient availability through body and faecal nutrient content. *Biol Fertil Soils* **14**: 71–5.
Tisdall JM and Oades JM. 1982. Organic matter and water-stable aggregates in soils. *J Soil Sci* **33**: 141–63.
Vierheilig H, Coughlan AP, Wyss U, and Piché Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl Environ Microbiol* **64**: 5004–7.
Wallenstein MD, Myrold DD, Firestone M, and Voytek M. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. *Ecol Appl* **16**: 2143–52.
Wu H, Lu M, Lu X, *et al.* 2015. Interactions between earthworms and mesofauna has no significant effect on emissions of CO2 and N2O from soil. *Soil Biol Biochem* **88**: 294–7.

6. GENERAL DISCUSSION AND CONCLUSIONS

Advances in molecular technology in recent years have allowed for the collection of fine-scale data about microbial community composition and functions. However, microbial ecological theory has not caught up with the quantity and quality of microbial community data being produced and despite broad application of these technologies to various environmental systems, characterization of microbial life in riverine and riparian habitats remains patchy. This doctoral work aimed to fill some of the existing knowledge gaps in this sphere through theoretical, field-based and experimental studies at the catchment, sub-catchment and biotic scales, respectively. While unique ecological processes were investigated in each of these works, they are conceptually linked: microbial-driven ecosystem functions depend on the trait space and abundances of the microbial taxa comprising a local community (Figure 6.1).

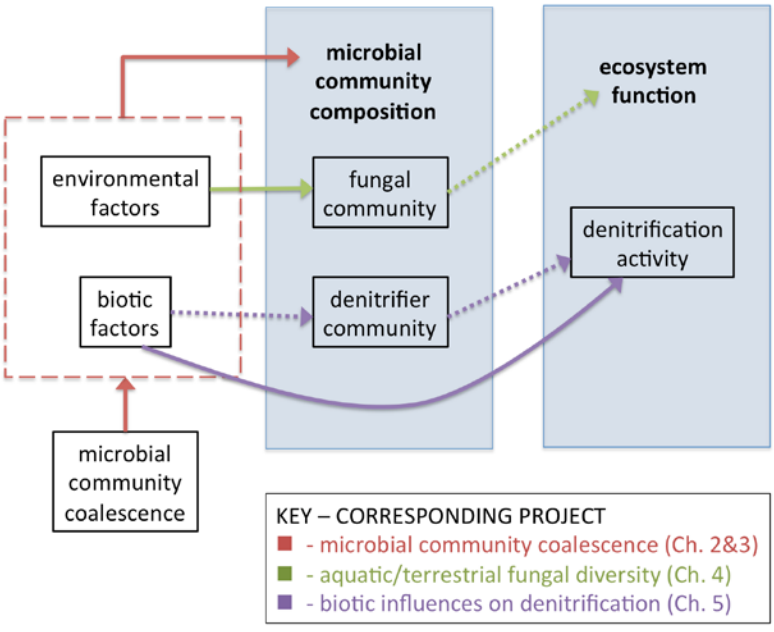

Figure 6.1. Conceptual overview of linkages between PhD works
The different colors represent three major lines of investigation (theoretical = pink, field-based = green, experimental microcosm study = purple). Solid arrows represent hypotheses tested in each study and dotted arrows represent implications of the results of the given studies.

6.1 Microbial Biodiversity

The study of biodiversity patterns is a central concern of community ecology. Community ecological theory was largely developed in communities of macro-organisms and thus has some limitations in its ability to explain microbial biodiversity patterns. Some processes that frequently occur in microbial systems, such as the mixing of previous distinct entire communities, are absent in macro-systems. We investigated the influence of microbial community coalescence, a recently proposed microbial ecological concept (Rillig *et al.* 2015), on lotic and riparian microbial community assembly through a review of field studies found evidence for the occurrence of this process in several riverine compartments (Chapter 2). In addition to theoretical development, we also conducted a study to investigate biodiversity patterns in the field (Chapter 4). General lotic fungal diversity is grossly understudied, particularly in comparison to terrestrial systems, lotic bacterial communities and the fungal functional groups.

Though not studied explicitly, community coalescence may have had an effect on the fungal community patterns that we observed in the field. We found that baseflow (i.e. the proportion of stream flow deriving from inputs of groundwater) explained some variation between observed fungal communities (Chapter 4). Upwelling flows of groundwater contain a microbial community unique from surface water and can also alter the local resource environment – groundwater typically has lower levels of dissolved O_2 and DOC and higher levels of inorganic nitrogen than surface water (Hendricks 1993). In Chalk streams with high baseflow values, the frequent mixing of groundwater communities with surface water communities, as well as the influence of groundwater chemistry on the benthic resource environment, may well have shaped the fungal communities that we observed. We also observed some fungal taxa typically associated with soil and livestock to be abundant in streams with lower baseflow values. In those streams, overland flow and throughflow contribute largely to stream flow and carried with them terrestrial fungal communities; some those taxa may have then proliferated in benthic sediments.

6.2 Microbial biodiversity and ecosystem function

Characterizing microbial biodiversity patterns may provide insight into ecosystem functioning. Reviews of biodiversity-ecosystem functioning (BEF) studies in stream ecosystems report that community composition (i.e. the type of taxa and trait space) rather than species richness (i.e. the number of different taxa) is the most important determinant of ecosystem functioning (Covich *et al.* 2004; Lecerf and Richardson 2007). Functional redundancy (i.e. multiple taxa with the ability to carry out the same function) is often invoked as an argument against the existence of strong BEF relationships; however, this was not observed in an experiment across multiple freshwater bacterial communities that observed a decrease in function with reduced diversity (Delgado-Baquerizo *et al.* 2016). This result points to the importance of collecting data about microbial biodiversity patterns. We investigated benthic fungal diversity and found distinct communities to inhabit sites of differing geology (Chapter 4). This work serves as one of very few studies reporting general lotic fungal biodiversity patterns; such data is critical to inform management strategies that maintain ecosystem functioning, particularly in such threatened ecosystems as rivers (Dudgeon *et al.* 2006).

We studied the influence of microarthropods (Collembola) on a process that contributes to the ecosystem function of nitrogen cycling, denitrification (Chapter 5). We observed higher fractional N_2O emissions in treatments containing these organisms. We expected that collembolan activity would stimulate the release of carbon and nutrients, leading to differences in denitrification activity in these treatments. While we did observe treatment differences, soil carbon and nitrogen levels were the same as or lower than the control and this hypothesis was not supported. Thus it is likely that the differences are attributable to indirect microarthopod effects on the denitrifier community, for example, through grazing on fungal hyphae leading to shifts in the fungal community. A strong BEF relationship has been observed for denitrifying microbial communities (Philippot *et al.* 2013), therefore it is important to study controls on this functional community to understand potential changes in denitrification activity. In riparian zones, which are an important source of N_2O emissions (Bouwman *et al.* 2013), denitrifying communities may be shaped by microbial community coalescence (Chapter 2) in addition to soil biota.

Flooding in these zones leading to an intermingling of surface water and riparian soil communities, and a recent study has shown the ability of aquatic taxa to persist in the soil environment following a simulated flooding event (Röhl *et al.* 2017). The degree to which the aquatic denitrifier community influences the riparian denitrifier community may be connected to community cohesion (Chapter 3): a high degree of cohesion among the riparian denitrifier community could reduce the degree to which members of the surface water community can establish and persist following the mixing event.

6.3 Conclusions and Outlook

This doctoral work contributes to the development of a conceptual ecological framework that could explain variability in observed lotic microbial communities, provides results from a field study investigating the under-studied communities of general lotic fungi and reports results from a manipulative study about biotic controls on denitrification activity. As riverine systems are simultaneously vital for ecosystem function and highly threatened by anthropogenic activity, there is an urgent need for fundamental knowledge of lotic biodiversity patterns and their relationship with function to inform conservation and restoration efforts.

REFERENCES

Altermatt F. 2013. Diversity in riverine metacommunities: A network perspective. *Aquat Ecol* **47**: 365–77.

Bärlocher F. 1987. Aquatic hyphomycete spora in 10 streams of New Brunswick and Nova Scotia. *Can J Bot* **65**: 76–9.

Bärlocher F and Corkum M. 2003. Nutrient enrichment overwhelms diversity effects in leaf decomposition by stream fungi. *Oikos* **101**: 247–52.

Bärlocher F, Seena S, Wilson KP, and Dudley Williams D. 2008. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshw Biol* **53**: 368–79.

Battin TJ, Besemer K, Bengtsson MM, *et al.* 2016. The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol* **14**: 251–63.

Battin TJ, Kaplan L a., Findlay S, *et al.* 2009. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat Geosci* **2**: 595–595.

Beaulieu JJ, Tank JL, Hamilton SK, *et al.* 2011. Nitrous oxide emission from denitrification in stream and river networks. *Proc Natl Acad Sci* **108**: 214–9.

Bender SF, Plantenga F, Neftel A, *et al.* 2014. Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. *ISME J* **8**: 1336–45.

Besemer K. 2015. Biodiversity, community structure and function of biofilms in stream ecosystems. *Res Microbiol* **166**: 774–81.

Besemer K, Singer G, Quince C, *et al.* 2013. Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proc R Soc B Biol Sci* **280**: 20131760–20131760.

Bouwman AF, Beusen AHW, Griffioen J, *et al.* 2013. Global trends and uncertainties in terrestrial denitrification and N2O emissions. *Philos Trans R Soc B Biol Sci* **368**: 20130112–20130112.

Boz B, Mizanur Rahman M, Bottegal M, *et al.* 2013. Vegetation, soil and hydrology management influence denitrification activity and the composition of nirK-type denitrifier communities in a newly afforested riparian buffer. *N Biotechnol* **30**: 675–84.

Braker G and Conrad R. 2011. Diversity, structure, and size of N2O-producing microbial communities in soil—what matters for their functioning? In: Advanced in Applied Microbiology, Volume 75.

Clivot H, Cornut J, Chauvet E, *et al.* 2014. Leaf-associated fungal diversity in acidified streams: Insights from combining traditional and molecular approaches. *Environ Microbiol* **16**: 2145–56.

Cole JJ, Prairie YT, Caraco NF, *et al.* 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10**: 171–84.

Covich AP, Austen MC, Bärlocher F, *et al.* 2004. The Role of Biodiversity in the Functioning of Freshwater and Marine Benthic Ecosystems. *Bioscience* **54**: 767.

Crump BC, Amaral-Zettler L a, and Kling GW. 2012. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *ISME J* **6**: 1629–39.

Dang CK, Chauvet E, and Gessner MO. 2005. Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. *Ecol Lett* **8**: 1129–37.

Danger M, Cornut J, Elger A, and Chauvet E. 2012. Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa. *Freshw Biol* **57**: 1017–30.

Delgado-Baquerizo M, Giaramida L, Reich PB, *et al.* 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. *J Ecol* **104**: 936–46.

Drenovsky RE, Vo D, Graham KJ, and Scow KM. 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microb Ecol* **48**: 424–30.

Droppo IG, Leppard GG, Flannigan DT, and Liss SN. 1997. The freshwater floc: A functional

relationship of water and organic and inorganic floc constituents affecting suspended sediment properties. *Water, Air, Soil Pollut* **99**: 43–53.
Dudgeon D, Arthington AH, Gessner MO, *et al.* 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol Rev* **81**: 163.
Fierer N, Morse JL, Berthrong ST, *et al.* 2007. Environmental Controls on the Landscape-Scale Biogeography of Stream Bacterial Communities. *Ecology* **88**: 2162–73.
Freimann R, Bürgmann H, Findlay SEG, and Robinson CT. 2015. Hydrologic linkages drive spatial structuring of bacterial assemblages and functioning in alpine floodplains. *Front Microbiol* **6**: 1–15.
Geraldes P, Pascoal C, and Cássio F. 2012. Effects of increased temperature and aquatic fungal diversity on litter decomposition. *Fungal Ecol* **5**: 734–40.
Harner MJ, Opitz N, Geluso K, *et al.* 2011. Arbuscular mycorrhizal fungi on developing islands within a dynamic river floodplain: An investigation across successional gradients and soil depth. *Aquat Sci* **73**: 35–42.
Harrop BL, Marks JC, and Watwood ME. 2009. Early bacterial and fungal colonization of leaf litter in Fossil Creek , Arizona. *J North Am Benthol Soc* **28**: 383–96.
Helton AM, Poole GC, Meyer JL, *et al.* 2011. Thinking outside the channel: modeling nitrogen cycling in networked river ecosystems. *Front Ecol Environ* **9**: 229–38.
Hendricks SP. 1993. Microbial Ecology of the Hyporheic Zone: A Perspective Integrating Hydrology and Biology. *J North Am Benthol Soc* **12**: 70–8.
Hieber M and Gessner MO. 2002. Contribution of Stream Detrivores, Fungi, and Bacteria To Leaf Breakdown Based on Biomass Estimates. *Ecology* **83**: 1026–38.
Hullar MAJ, Kaplan LA, and Stahl DA. 2006. Recurring Seasonal Dynamics of Microbial Communities in Stream Habitats. *Appl Environ Microbiol* **72**: 713–22.
Krauss G-J, Solé M, Krauss G, *et al.* 2011. Fungi in freshwaters: ecology, physiology and biochemical potential. *FEMS Microbiol Rev* **35**: 620–51.
Langhans SD, Tiegs SD, Gessner MO, and Tockner K. 2008. Leaf-decomposition heterogeneity across a riverine floodplain mosaic. *Aquat Sci* **70**: 337–46.
Lansdown K, McKew BA, Whitby C, *et al.* 2016. Importance and controls of anaerobic ammonium oxidation influenced by riverbed geology. *Nat Geosci* **9**: 357–60.
Lecerf A and Chauvet E. 2008. Diversity and functions of leaf-decaying fungi in human-altered streams. *Freshw Biol* **53**: 1658–72.
Lecerf A and Richardson JS. 2007. Biodiversity-Ecosystem Function Research: Insights Gained from Streams. *River Res Appl* **26**: 45–54.
Liu J, Wang J, Gao G, *et al.* 2015. Distribution and diversity of fungi in freshwater sediments on a river catchment scale. *Front Microbiol* **6**: 1–12.
Maeda K, Spor A, Edel-Hermann V, *et al.* 2015. N2O production, a widespread trait in fungi. *Sci Rep* **5**: 9697.
Manerkar M a, Seena S, and Bärlocher F. 2008. Q-RT-PCR for Assessing Archaea, Bacteria, and Fungi During Leaf Decomposition in a Stream. *Microb Ecol* **56**: 467–73.
Mardhiah U, Caruso T, Gurnell A, and Rillig MC. 2014. Just a matter of time: Fungi and roots significantly and rapidly aggregate soil over four decades along the Tagliamento River, NE Italy. *Soil Biol Biochem* **75**: 133–42.
Mardhiah U, Caruso T, Gurnell A, and Rillig MC. 2016. Arbuscular mycorrhizal fungal hyphae reduce soil erosion by surface water flow in a greenhouse experiment. *Appl Soil Ecol* **99**: 137–40.
Marhan S, Auber J, and Poll C. 2015. Additive effects of earthworms, nitrogen-rich litter and elevated soil temperature on N2O emission and nitrate leaching from an arable soil. *Appl Soil Ecol* **86**: 55–61.
Mermillod-Blondin F, Gaudet JP, Gerino M, *et al.* 2004. Relative influence of bioturbation and predation on organic matter processing in river sediments: A microcosm experiment.

Freshw Biol **49**: 895–912.
Miura A and Urabe J. 2015a. Riparian land cover and land use effects on riverine epilithic fungal communities. *Ecol Res* **30**: 1047–55.
Miura A and Urabe J. 2015b. Spatial and seasonal changes in species diversity of epilithic fungi along environmental gradients of a river. *Freshw Biol* **60**: 673–85.
Muehlbauer JD, Collins SF, Doyle MW, and Tockner K. 2014. How wide is a stream? Spatial extent of the potential "stream signature" in terrestrial food webs using meta-analysis. *Ecology* **95**: 44–55.
Naiman RJ, Decamps H, and McClain ME. 2005. Riparia: Ecology, Conservation, and Management of Streamside Communities (N Maragioglio and K Sonnack, Eds). Elsevier Academic Press.
Philippot L. 2002. Denitrifying genes in bacterial and Archaeal genomes. *Biochim Biophys Acta - Gene Struct Expr* **1577**: 355–76.
Philippot L, Spor A, Hénault C, *et al.* 2013. Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* **7**: 1609–19.
Pinay G, Black VJ, Planty-Tabachhi AM, *et al.* 2000. Geomorphic control of denitrificaiton in large river floodplain soils. *Biogeochemistry* **50**: 163–82.
Read DS, Gweon HS, Bowes MJ, *et al.* 2015. Catchment-scale biogeography of riverine bacterioplankton. *ISME J* **9**: 516–26.
Rillig MC, Antonovics J, Caruso T, *et al.* 2015. Interchange of entire communities: microbial community coalescence. *Trends Ecol Evol* **30**: 470–6.
Risse-Buhl U, Trefzger N, Seifert AG, *et al.* 2012. Tracking the autochthonous carbon transfer in stream biofilm food webs. *FEMS Microbiol Ecol* **79**: 118–31.
Robertson AI, Bunn SE, Boon PI, and Walker KF. 1999. Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Mar Freshw Res* **50**: 813.
Röhl O, Graupner N, Peršoh D, *et al.* 2017. Flooding Duration Affects the Structure of Terrestrial and Aquatic Microbial Eukaryotic Communities. *Microb Ecol*.
Rovelli L, Attard KM, Binley A, *et al.* 2017. Reach-scale river metabolism across contrasting sub-catchment geologies: Effect of light and hydrology. *Limnol Oceanogr*.
Rubin MA and Leff LG. 2007. Nutrients and other abiotic factors affecting bacterial communities in an Ohio River (USA). *Microb Ecol* **54**: 374–83.
Ruiz-González C, Niño-García JP, and Giorgio P a. del. 2015. Terrestrial origin of bacterial communities in complex boreal freshwater networks (N Fierer, Ed). *Ecol Lett* **18**: 1198–206.
Savio D, Sinclair L, Ijaz UZ, *et al.* 2015. Bacterial diversity along a 2600 km river continuum. *Environ Microbiol* **17**: 4994–5007.
Seo DC and DeLaune RD. 2010. Fungal and bacterial mediated denitrification in wetlands: Influence of sediment redox condition. *Water Res* **44**: 2441–50.
Sgouridis F, Heppell CM, Wharton G, *et al.* 2011. Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in a temperate re-connected floodplain. *Water Res* **45**: 4909–22.
Shrestha J, Niklaus P a., Pasquale N, *et al.* 2014. Flood pulses control soil nitrogen cycling in a dynamic river floodplain. *Geoderma* **228–229**: 14–24.
Soininen J, Bartels P, Heino J, *et al.* 2015. Toward More Integrated Ecosystem Research in Aquatic and Terrestrial Environments. *Bioscience* **65**: 174–82.
Stutter MI and Richards S. 2012. Relationships between Soil Physicochemical, Microbiological Properties, and Nutrient Release in Buffer Soils Compared to Field Soils. *J Environ Qual* **41**: 400.
Tatariw C, Chapman EL, Sponseller RA, *et al.* 2013. Denitrification in a large river: consideration of geomorphic controls on microbial activity and community structure. *Ecology* **94**: 2249–62.
Tomasek A, Staley C, Wang P, *et al.* 2017. Increased denitrification rates associated with shifts in

prokaryotic community composition caused by varying hydrologic connectivity. *Front Microbiol* **8**: 1–12.
Trimmer M, Grey J, Heppell CM, *et al.* 2012. River bed carbon and nitrogen cycling: State of play and some new directions. *Sci Total Environ* **434**: 143–58.
Wallenstein MD, Myrold DD, Firestone M, and Voytek M. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. *Ecol Appl* **16**: 2143–52.
Widder S, Besemer K, Singer G a, *et al.* 2014. Fluvial network organization imprints on microbial co-occurrence networks. *Proc Natl Acad Sci U S A* **111**: 1–6.
Wilson JS, Baldwin DS, Rees GN, and Wilson BP. 2011. The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil. *River Res Appl* **27**: 213–25.
Yu S-X, Pang Y-L, Wang Y-C, *et al.* 2017. Spatial variation of microbial communities in sediments along the environmental gradients from Xiaoqing River to Laizhou Bay. *Mar Pollut Bull* **120**: 90–8.

APPENDICES

Appendix A2: Chapter 2

Table A2.1 Field studies reviewed

	First Author	Title	Journal	Year	Organism	Compartment or Event
1	Lin	Spatial and temporal dynamics of the microbial community in the Hanford unconfined aquifer	ISME	2012	multiple	aquifer
2	Ruiz-González	Effects of large river dam regulation on bacterioplankton community structure	FEMS Microbiology Ecology	2013	bacteria	dam
3	Colas	Dam-associated multiple-stressor impacts on fungal biomass and richness reveal the initial signs of ecosystem functioning impairment	Ecological Indicators	2016	fungi	dam
4	Crump and Baross	Archaeaplankton in the Columbia River, its estuary and the adjacent coastal ocean, USA	FEMS Microbiology Ecology	2000	archaea	estuary
5	Campbell and Kirchman	Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient	ISME	2012	bacteria	estuary
6	Crump	Microbial Biogeography along an Estuarine Salinity Gradient : Combined Influences of Bacterial Growth and Residence Time	Applied and Environmental Microbiology	2004	bacteria	estuary
7	Crump	Phylogenetic Analysis of Particle-Attached and Free-Living Bacterial Communities in the Columbia River, Its Estuary, and the Adjacent Coastal Ocean	Applied and Environmental Microbiology	1999	bacteria	estuary
8	Fortunato	Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient	ISME	2012	bacteria	estuary
9	Fagervold	River organic matter shapes microbial communities in the sediment of the Rhône prodelta	ISME	2014	bacteria	estuary
10	Fortunato	Microbial gene abundance and expression patterns across a river to ocean salinity gradient	PLoS One	2015	bacteria	estuary

11	Fortunato	Bacterioplankton Community Variation Across River to Ocean Environmental Gradients	Microbial Ecology	2011	bacteria	estuary
12	De Almeida	Yeast community survey in the Tagus estuary	FEMS Microbiology Ecology	2005	fungi	estuary
13	Gadanho	Application of temperature gradient gel electrophoresis to the study of yeast diversity in the estuary of the Tagus river, Portugal.	FEMS yeast research	2004	fungi	estuary
14	Bouvier	Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries	Limnology and Oceanography	2002	multiple	estuary
15	Mosier and Francis	Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary	Environmental Microbiology	2008	multiple	estuary
16	Webster	Archaeal community diversity and abundance changes along a natural salinity gradient in estuarine sediments	FEMS Microbiology Ecology	2015	multiple	estuary
17	Cousin	Flavobacterial community structure in a hardwater rivulet and adjacent forest soil, Harz Mountain, Germany	Current Microbiology	2009	bacteria	floodplain
18	Freimann	Hydrologic linkages drive spatial structuring of bacterial assemblages and functioning in alpine floodplains	Frontiers in Microbiology	2015	bacteria	floodplain
19	Harner	Heterogeneity in mycorrhizal inoculum potential of flood-deposited sediments	Aquatic Sciences	2009	fungi	floodplain
20	Whitfield	Relationships between soil heavy metal concentration and mycorrhizal colonisation in Thymus polytrichus in northern England	Mycorrhiza	2004	fungi	floodplain
21	Rinklebe	Floodplain soils at the Elbe river, Germany, and their diverse microbial biomass	Archives of Agronomy and Soil Science	2008	multiple	floodplain
22	Rinklebe	Microbial diversity in three floodplain soils at the Elbe River (Germany)	Soil Biology and Biochemistry	2006	multiple	floodplain
23	Stutter	Relationships between Soil Physicochemical, Microbiological Properties, and Nutrient Release in Buffer Soils Compared to Field Soils	Journal of Environmental Quality	2012	multiple	floodplain
24	Unger	Flooding effects on soil microbial communities	Applied Soil Ecology	2009	multiple	floodplain
25	Besemer	Headwaters are critical reservoirs of microbial diversity for fluvial networks	Proceedings of the Royal Society B	2013	bacteria	headwaters
26	Read	Catchment-scale biogeography of riverine bacterioplankton	ISME	2015	bacteria	headwaters
27	Ruiz-Gonzalez	Terrestrial origin of bacterial communities in complex boreal freshwater networks	Ecology Letters	2015	bacteria	headwaters

28	Savio	Bacterial diversity along a 2600 km river continuum	Environmental Microbiology	2015	bacteria	headwaters
29	Crump	Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils	ISME	2012	multiple	headwaters
30	Staley	Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River	Journal of Applied Microbiology	2013	bacteria	headwaters
31	Danger	Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa	Freshwater Biology	2012	fungi	hypoheic
32	Brablcová	Methanogenic archaea diversity in hyporheic sediments of a small lowland stream	Anaerobe	2015	archaea	hyporheic
33	Febria	Bacterial community dynamics in the hyporheic zone of an intermittent stream	ISME	2012	bacteria	hyporheic
34	Lowell	Habitat heterogeneity and associated microbial community structure in a small-scale floodplain hyporheic flow path	Microbial Ecology	2009	bacteria	hyporheic
35	Stegen	Groundwater–surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover	Nature Communications	2016	bacteria	hyporheic
36	Feris	Seasonal Dynamics of Shallow-Hyporheic-Zone Microbial Community Structure along a Heavy-Metal Contamination Gradient	Applied and Environmental Microbiology	2004	bacteria	hyporheic
37	Feris	Differences in Hyporheic-Zone Microbial Community Structure along a Heavy-Metal Contamination Gradient	Applied and Environmental Microbiology	2003	bacteria	hyporheic
38	Hamonts	Determinants of the microbial community structure of eutrophic, hyporheic river sediments polluted with chlorinated aliphatic hydrocarbons	FEMS Microbiology Ecology	2014	bacteria	hyporheic
39	Cornut	Effect of acidification on leaf litter decomposition in benthic and hyporheic zones of woodland streams	Water Research	2012	fungi	hyporheic
40	Cornut	Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams	Freshwater Biology	2010	fungi	hyporheic
41	Clivot	Leaf-associated fungal diversity in acidified streams: Insights from combining traditional and molecular approaches	Environmental Microbiology	2014	fungi	litter
42	Nikolcheva	Determining Diversity of Freshwater Fungi on Decaying Leaves: Comparison of Traditional and Molecular Approaches	Applied and Environmental Microbiology	2003	fungi	litter

43	Sridhar	Aquatic hyphomycetes on leaf litter in and near a stream in Nova Scotia, Canada	Mycological Research	1993	fungi	litter
44	Bärlocher	Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams	Freshwater Biology	2002	fungi	litter
45	Bärlocher	Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes	Freshwater Biology	2008	fungi	litter
46	Batista, D	Impacts of warming on aquatic decomposers along a gradient of cadmium stress	Environmental Pollution	2012	fungi	litter
47	Baudoin, J. M.	Elevated aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf-litter breakdown	Microbial Ecology	2008	fungi	litter
48	Bruder	Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency	Functional Ecology	2011	fungi	litter
49	Cai	Variation between freshwater and terrestrial fungal communities on decaying bamboo culms.	Antonie van Leeuwenhoek	2006	fungi	litter
50	Das	Diversity of Fungi, Bacteria, and Actinomycetes on Leaves Decomposing in a Stream	Applied and Environmental Microbiology	2007	fungi	litter
51	Das	Fungal communities on decaying leaves in streams: A comparison of two leaf species	Mycological Progress	2008	fungi	litter
52	Duarte	High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams.	Microbial Ecology	2008	fungi	litter
53	Duarte	Stream-dwelling fungal decomposer communities along a gradient of eutrophication unraveled by 454 pyrosequencing	Fungal Diversity	2015	fungi	litter
54	Fernandes	Higher temperature reduces the effects of litter quality on decomposition by aquatic fungi	Freshwater Biology	2012	fungi	litter
55	Ferreira	Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates	Oecologia	2006	fungi	litter
56	Fryar	The Influence of Competition between Tropical Fungi on wood colonization in Streams.	Microbial ecology	2001	fungi	litter
57	Gulis	Leaf litter decompositioin and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream	Freshwater Biology	2003	fungi	litter
58	Harrop	Early bacterial and fungal colonization of leaf litter in Fossil Creek , Arizona	Journal of the North American Benthological Society	2009	fungi	litter

59	Kane	Fungi colonising and sporulating on submerged wood in the River Severn, UK	Fungal Ecology	2002	fungi	litter
60	Lecerf	Diversity and functions of leaf-decaying fungi in human-altered streams	Freshwater Biology	2008	fungi	litter
61	Lecerf	Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems	Oecologia	2005	fungi	litter
62	Manerkar	Q-RT-PCR for Assessing Archaea, Bacteria, and Fungi During Leaf Decomposition in a Stream	Microbial Ecology	2008	fungi	litter
63	Nikolcheva	Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence	Freshwater Biology	2005	fungi	litter
64	Nikolcheva	Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream	Mycological Progress	2004	fungi	litter
65	Nikolcheva	Determining Diversity of Freshwater Fungi on Decaying Leaves : Comparison of Traditional and Molecular Approaches	Applied and Environmental Microbiology	2003	fungi	litter
66	Pascoal	Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River	Applied and Environmental Microbiology	2004	fungi	litter
67	Seena	Fungal diversity during leaf decomposition in a stream assessed through clone libraries	Fungal Diversity	2008	fungi	litter
68	Sridhar	The role of early fungal colonizers in leaf-litter decomposition in portuguese streams impacted by agricultural runoff	International Review of Hydrobiology	2009	fungi	litter
69	Suberkropp	Regulation of Leaf Breakdown by Fungi in Streams: Influences of Water Chemistry	Ecology	1995	fungi	litter
70	Tolkkinen	Multi-stressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress	Ecology	2015	fungi	litter
71	Baldy	Microbial dynamics associated with leaves decomposing in the mainstream and floodplain pond of a large river	Aquatic Microbial Ecology	2002	fungi	litter
72	Fernández	Effects of fungicides on decomposer communities and litter decomposition in vineyard streams	Science of the Total Environment	2015	fungi	litter
73	Kelly	Alteration of microbial communities colonizing leaf litter in a temperate woodland stream by growth of trees under conditions of elevated atmospheric CO2	Applied and Environmental Microbiology	2010	multiple	litter
74	Kominoski	Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species?	Oikos	2009	multiple	litter

75	Frossard	Litter supply as a driver of microbial activity and community structure on decomposing leaves: A test in experimental streams	Applied and Environmental Microbiology	2013	multiple	litter
76	Cebron	Denaturing Gradient Gel Electrophoretic Analysis of Ammonia-Oxidizing Bacterial Community Structure in the Lower Seine River: Impact of Paris Wastewater Effluents	Applied and Environmental Microbiology	2004	bacteria	wastewater
77	García-Armisen	Seasonal variations and resilience of bacterial communities in a sewage polluted urban river	PLoS ONE	2014	bacteria	wastewater
78	Drury	Wastewater treatment effluent reduces the abundance and diversity of benthic bacterial communities in urban and suburban rivers	Applied and Environmental Microbiology	2013	bacteria	wastewater
79	Wakelin	Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow	Applied and Environmental Microbiology	2008	bacteria	wastewater
80	Cudowski	Aquatic fungi in relation to the physical and chemical parameters of water quality in the Augustów Canal	Fungal Ecology	2015	fungi	wastewater

Appendix A4: Chapter 4

A4.1 Fungal communities inhabiting different patch types

An additional PCoA was conducted on samples taken from vegetated, unvegetated and marginal bank sediments at two of the nine sites to determine if there was any influence of patch type (i.e. niche; Figure S2). The amount of variance explained by the first two PCoA axes was low (17.12%). Communities do not cluster by patch type, but do cluster by site. There is some degree of separation between patch types in the chalk samples, where fungal communities in the vegetated patches appear to be somewhat intermediate between those in the unvegetated sediment and marginal bank sediment patches. At the greensand site, fungal communities from different patch types are not at all separated.

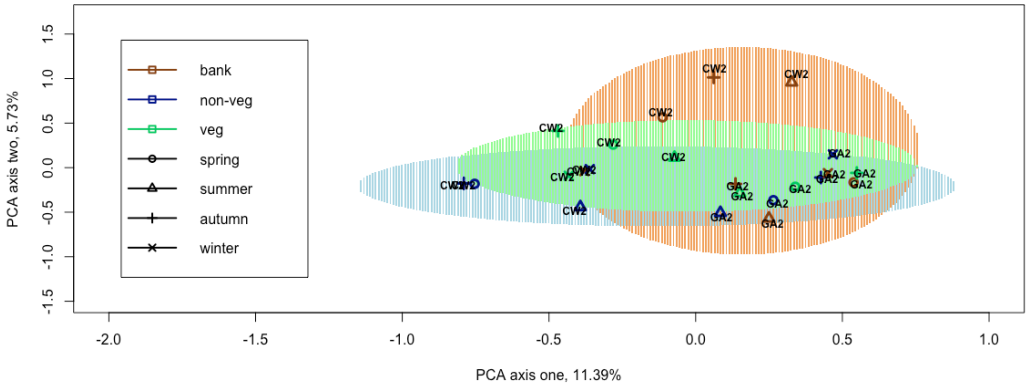

Figure A4.1 PCoA of fungal communities inhabiting different patch types
Patch types: vegetated, non-vegetated and marginal bank sediments, at two of the sampling sites across 4 seasons.

A4.2 Assessment of collinearity of predictor variables

A range of additional predictor variables were measured/assessed, including percent agricultural land use, riparian tree diversity on both banks of a 30m reach, stream order and catchment size. These predictors were all found to be highly collinear with BFIHOST and therefore were not included in our models.

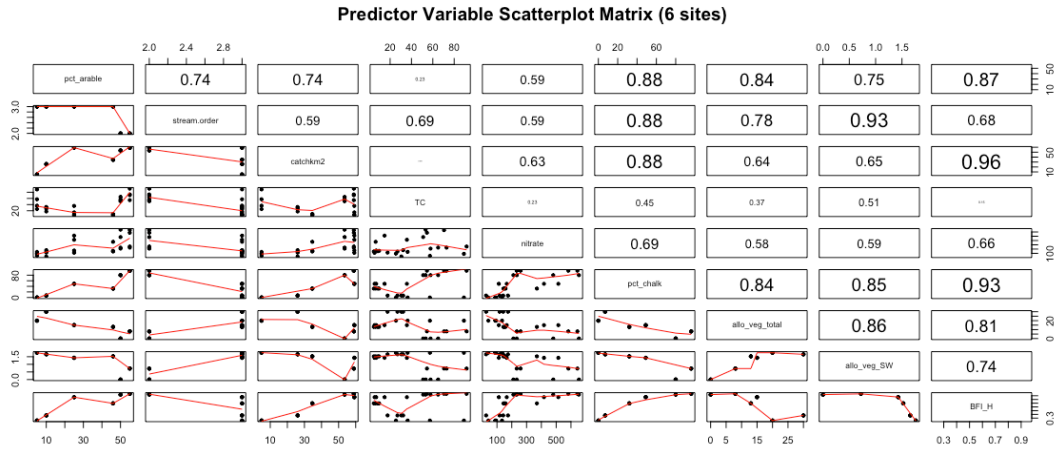

Figure A4.2 Scatterplot showing relationships between various predictor variables

A4.3 Riparian tree diversity

Table A4.1 Riparian tree diversity along both banks of a 30m river reach

Common name	Latin name	AS1	AS2	GN1	GA2	CE1	CW2
Common ash	Fraxinus excelsior	4	2	3	1	4	0
Hawthorne	Crataegus monogyna	5	5	0	5	1	0
Goat willow	Salix caprea	0	3	0	2	0	0
Field maple	Acer campestre	0	9	0	0	1	0
Buckthorn	Rhamnus cathartica	4	9	0	0	0	0
Hazel	Corylus avellana	0	1	3	0	0	0
Common Alder	Alnus glutinosa	1	0	1	0	0	0
Elder	Sambucus nigra	0	0	2	2	0	0
Crack willow	Salix fragilis	0	0	4	1	0	0
Nettle	Urtica dioica	0	0	many	0	0	0
Willow (spp unknown)	Salix spp	0	0	0	4	1	0
Blackberry	Rubus sp	1	0	0	0	1	0
Damsons	Prunus domesticus	4	0	0	0	0	0
English oak	Quercus robur	1	1	0	0	0	0
	Total plants	20	30	13	15	8	0
	Richness	7	7	6	6	5	0

A4.4 Indicator species analysis

We further investigated the species that were specific to each geology by looking at the species identity of indicators significant at the p = 0.005 level. (Assessing all indicator species significant at p = 0.05 was precluded by the number of indicators at this level – a total of 389.) Nearly 50% of the indicator species at the clay sites were aquatic hyphomycete taxa, or the classic riverine allochthonous litter decomposers. This is a sensible result given that there is also the highest density and diversity of riparian trees at the clay sites. Three of four indicator species at the sand sites were associated with terrestrial habitats – one of four was Onygenales, taxa associated with the skin and hair of mammals, and one Mortierella, a typical soil saprotroph.

Table A4.2. Identity of the indicator species of the various geologies significant at p = 0.005

Geology	Phylum	Class	Order	Family	Genus	Species
Chalk	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis_13	Phoma	Phoma brasiliensis
	Ascomycota	Unclassified Ascomycota	Unclassified Ascomycota	Unclassified Ascomycota	Unclassified Ascomycota	Unclassified Ascomycota
Clay	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Leotiomycetes	Helotiales	Incertae sedis_2	Tetracladium	Unclassified Tetracladium
	Ascomycota	Dothideomycetes	Incertae sedis_8	Pseudeurotiaceae	Pseudogymnoascus	Pseudogymnoascus roseus
	Ascomycota	Sordariomycetes	Sordariomycetes unidentified	Sordariomycetes unidentified_1	Sordariomycetes unidentified_1	Sordariomycetes sp
	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Lasiosphaeriaceae unidentified	Lasiosphaeriaceae sp

Geology	Phylum	Class	Order	Family	Genus	Species
Clay, cntd.	Ascomycota	Dothideomycetes	Incertae sedis_8	Pseudeurotiaceae	Pseudeurotium	Pseudeurotium hygrophilum
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Dothideomycetes	Incertae sedis_8	Pseudeurotiaceae	Pseudeurotium	Pseudeurotium hygrophilum
	Ascomycota	Dothideomycetes	Dothideomycetes unidentified	Dothideomycetes unidentified_1	Dothideomycetes unidentified_1	Dothideomycetes sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Leotiomycetes	Helotiales	Incertae sedis_2	Tetracladium	Unclassified Tetracladium
	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Cercophora	Unclassified Cercophora
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Flagellospora	Flagellospora sp
	Ascomycota	Leotiomycetes	Helotiales	Incertae sedis_2	Tetracladium	Unclassified Tetracladium
	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	Hypochnicium	Unclassified Hypochnicium
	Ascomycota	Dothideomycetes	Incertae sedis_8	Pseudeurotiaceae	Pseudeurotium	Pseudeurotium hygrophilum
	Ascomycota	Leotiomycetes	Helotiales	Incertae sedis_2	Cadophora	Unclassified Cadophora
Sand	Zygomycota	Incertae sedis_10	Mortierellales	Mortierellaceae	Mortierella	Mortierella sp
	Ascomycota	Sordariomycetes	Sordariomycetes unidentified	Sordariomycetes unidentified_1	Sordariomycetes unidentified_1	Sordariomycetes sp
	Ascomycota	Dothideomycetes	Dothideomycetes unidentified	Dothideomycetes unidentified_1	Dothideomycetes unidentified_1	Dothideomycetes sp

Geology	Phylum	Class	Order	Family	Genus	Species
Sand, cntd.	Ascomycota	Eurotiomycetes	Onygenales	Onygenales unidentified	Onygenales unidentified_1	Onygenales sp
Clay + Sand	Ascomycota	Dothideomycetes	Dothideomycetes unidentified	Dothideomycetes unidentified_1	Dothideomycetes unidentified_1	Dothideomycetes sp
	Ascomycota	Sordariomycetes	Sordariomycetes unidentified	Sordariomycetes unidentified_1	Sordariomycetes unidentified_1	Sordariomycetes sp
	Ascomycota	Sordariomycetes	Hypocreales	Incertae_sedis_3	Ilyonectria	Ilyonectria macrodidyma
	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	Nectria ramulariae

A4.5 Alternative ordination analysis

Because of the nature of our samples (both distinguished categorically by underlying geology and lying along a hydrological gradient), two ordination techniques appropriate for these circumstances were applied. Assuming that the sites truly belong in distinct categories based on geology, an unconstrained ordination technique would be appropriate, as was employed (Principal Coordinates Analysis; PCoA; Figure 4.2). However, a slight overlap between some of the greensand and chalk sites was observed in the PCoA. In the case that the hydrological gradient of baseflow is a better indicator of the fungal sediment habitat than the sites truly being distinguished by the underlying geology, a constrained ordination approach incorporating the baseflow index values would be a more appropriate statistical technique. Thus, a canonical correspondence analysis (CCA) was conducted, including hydrological and chemical predictor variables. The inclusion of predictor variables did not increase the explanatory power of the ordination: 26.78% of variance between communities was explained employing CCA. Therefore, the original PCoA analysis was utilized. The results of the CCA analysis are presented here for comparison (Figure A4.3).

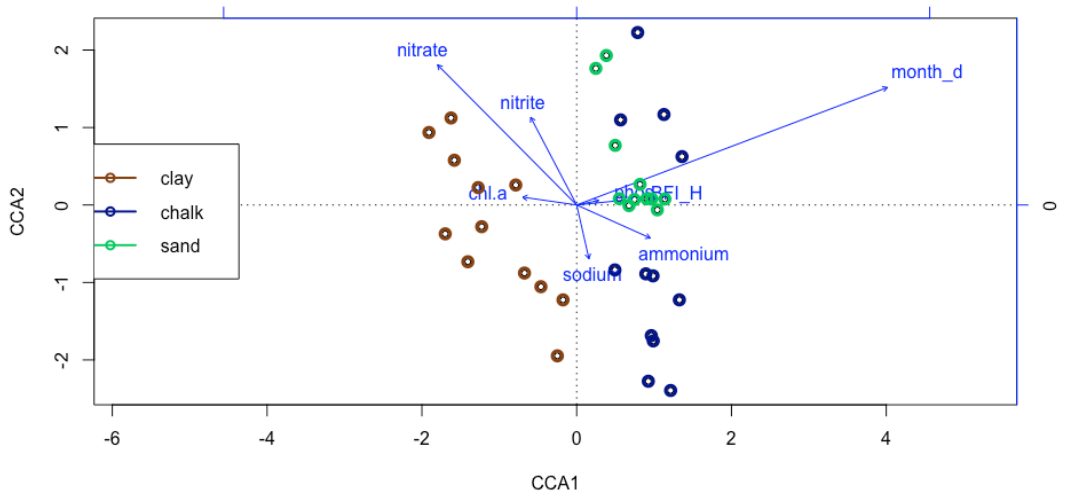

Figure A4.3 Sediment fungal communities; CCA