Microbial Diversity in Alpine Floodplains: Spatio-Temporal Factors Influencing Bacterial Communities and Ecosystem Functioning

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Summary

Microbes, such as heterotrophic bacteria, are crucial in the functional ecology of terrestrial and aquatic ecosystems, being the driving force behind metabolic processes, nutrient retention and cycling, and trophic links with secondary consumers. Bacterial biodiversity, including the actual genetic and functional spectrum, has only recently been investigated and knowledge about alpine lotic ecosystems and their local bacterial communities, in particular, is even scarcer. The ongoing climate-induced change in ecosystems is very likely to influence bacterial communities and consequently ecosystem functioning. To what extent shifts in ecosystem functioning due to future landscape transformation will be buffered by a potential resistance/resilience of bacterial communities is mostly unknown. Glaciated alpine floodplains provide excellent opportunities to test such fundamental ecological questions as they have a high degree of insularity, biotic endemicity and are sensitive to environmental change. In addition, these landscapes harbor structured, hydrologically interconnected and spatio-temporally heterogeneous landscape features such as different stream types or interspersed lakes. These features can provide additional mechanistic understanding of how structures within landscapes drive biodiversity and ecosystem functioning in space and time. Such knowledge could be extrapolated to similar ecosystems that potentially undergo future landscape changes induced by climate change and would improve models of ecosystem services.

The overall aim of the present dissertation is to add to the scarce knowledge about mechanisms driving bacterial community structure and function within glaciated alpine floodplains in a spatio-temporal context and to assess potential effects of climate induced changes within these landscapes. Several molecular biological methods, i.e. automated ribosomal intergenic spacer amplification (ARISA), catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) and enzymatic activity analysis in combination with a set of multivariate statistics were used to approach these questions. The first project was an extended seasonal sampling study that covered three hydrologically distinct periods and provided the large-scale context for the follow up studies. To cover a broad range of landscape features and add more generality to this survey, we incorporated three alpine floodplains differing in geology, their degree of deglaciation, the presence of lakes, and their physico-chemical characteristics. The second chapter of the thesis focused on smaller scale landscape interactions in the context of hydrology. Piezometers that served as incubation chambers for bacteria were installed at two locations within a glaciated valley, each representing a different array of morphological landscape structures. Different hydrologic periods were covered to assess the importance of spatial connectivity on formation and dynamics of bacterial communities and their related functions within riparian zone soils and in-stream hyporheic sediments. The third project assessed the impact of potential future shifts in water sources and altered nutritional states on hyporheic sediment bacterial communities and functions of these aquatic ecosystems. Here, mesocosm experiments were performed in which sediments from a glacial and groundwater channel were reciprocally transplanted and nutrients added. This experiment was repeated during three different seasons to cover potential temporal fluctuations within the sediment bacterial communities.

The results of the three studies revealed distinct bacterial community composition and functional differences among the catchments and different degrees of separation in relation to structure, function and their seasonality between glacial- and groundwater-fed stream types. Physico-chemical properties dictated bacterial structure and partially controlled their functioning. Groundwater systems were temporally more stable and showed higher degree of uncoupling between structure and function, indicating a greater prevalence of generalists. Community assemblages in the riparian zone and hyporheic sediments appeared to depend on the hydrological state of the system, thus landscape connectivity, and strategies of apparent bacterial taxa. Furthermore, the results showed that flow-mediated processes strongly influence bacterial functioning within alpine floodplains. Finally, results of transplanted bacterial communities suggest high resistance to an altered water resource or nutritional state. This was true for native glacial and groundwater communities with the latter being superior in terms of resistance. Surprisingly, there was a pronounced degree of functional plasticity/functional redundancy apparent within both bacterial communities during any season, as they quickly adapted functionally to new environmental and nutritional conditions.

In summary, the results presented in this thesis highlight the hierarchically structured, complex and partly interconnected factors influencing bacterial communities of alpine landscapes. Bacterial communities and related functions are likely to shift in concert with ongoing glacial retreat and the rapid changes in their eco-hydrological environment.

Zusammenfassung

Mikroorganismen, wie heterotrophe Bakterien, spielen eine entscheidende Rolle in der funktionellen Ökologie von terrestrischen und aquatischen Ökosystemen. Sie stellen die treibende Kraft hinter metabolischen Prozessen, Nährstoffrückhaltung und Wiederverwertung, wie auch die Verbindung mit höheren trophischen Stufen dar. Bakterielle Biodiversität, welche die genetische als auch funktionelle Vielfalt darstellt, ist erst seit relativ kurzer Zeit im Fokus der Wissenschaft. Dies gilt insbesondere für alpine Fliessgewässer und ihre bakteriellen Gemeinschaften.

Die durch den fortschreitenden Klimawandel induzierte Veränderung von Ökosystemen wird höchst wahrscheinlich die bakteriellen Gemeinschaften beeinflussen und konsequenterweise die mit ihnen verbundenen Ökosystemprozesse. Zu welchem Teil Veränderungen von Ökosystemfunktionen durch die zukünftigen Landschaftsveränderungen von einer potentiellen Resistenz/Anpassungsfähigkeit der bakteriellen Gemeinschaft abgefedert wird, ist mehrheitlich unbekannt. Vergletscherte alpine Überschwemmungsebenen bieten eine ausgezeichnete Möglichkeit, solchen fundamentalen ökologischen Fragen nachzugehen, da sie durch ein hohes Mass an Abgeschlossenheit, Endemismus und Sensitivität für Umweltveränderungen charakterisiert sind. Zusätzlich beinhalten sie strukturierte, hydrologisch verbundene, räumlich und zeitlich heterogene Landschaftsmerkmale, wie z.B. verschiedene Flusstypen oder zwischengeschaltete Stillgewässer. Die Erforschung dieser Aspekte kann zusätzliches Verständnis für die landschaftsgebundenen Faktoren generieren, welche die bakterielle und funktionelle Biodiversität beeinflussen. Dieses Wissen könnte man auf ähnliche Ökosysteme übertragen, die durch den Klimawandel beeinflusst werden und dadurch Modelle, welche Ökosystemdienstleistungen voraussagen, verbessern.

Das Hauptziel der hier präsentierten Dissertation ist es, das knappe Wissen über die Mechanismen, welche die bakteriellen Gemeinschaften und Funktionen innerhalb alpiner vergletscherter Überschwemmungsebenen in Raum und Zeit beeinflussen, substantiell zu erweitern und mögliche Auswirkungen zukünftiger Landschaftsveränderungen abzuschätzen. Diverse molekularbiologische Methoden, wie automated ribosomal intergenic spacer amplification (ARISA), catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) und die Analyse von Enzymaktivitäten, wurden dabei mittels verschiedener multivariaten Modellen ausgewertet, um die gestellten Fragen zu beantworten. Beim ersten Projekt handelte es sich um eine extensive saisonale Feldstudie, welche drei hydrologisch unterschiedliche Perioden abdeckte und den grossskalierten Hintergrund für die darauffolgenden Studien lieferte. Um eine möglichst grosse Bandbreite an Landschaftscharakteristiken abzudecken und die Allgemeingültigkeit dieser Studie zu erhöhen, untersuchten wir drei alpine Einzugsgebiete, welche sich in ihrem Grad der Deglaziation, der Geologie, dem Vorkommen von Seen und der allgemeinen physikochemischen Charakteristik unterscheiden. Das zweite Kapitel dieser Dissertation fokussierte sich auf kleinräumigere Landschaftsinteraktionen in Zusammenhang mit der Hydrologie. Piezometer dienten als Inkubationskammern für Bakterien und wurden an zwei Orten in einem Tal installiert, welches ein vielfältiges Spektrum morphologischer Landschaftstrukturen enthält. Perioden mit unterschiedlichen hydrologischen Bedingungen wurden gewählt, um die Wichtigkeit räumlicher Konnektivität für die Formung und Dynamik bakterieller Gemeinschaften und ihre Funktionen innerhalb der Uferzone und im hyporheischen Sediment zu ergründen. Das dritte Projekt untersuchte den Einfluss von potentiellen zukünftigen Veränderungen der Wasserressourcen und des Nährstoffstatus in den hyporheischen Sedimenten auf Struktur und Funktion der Bakteriengemeinschaften. Dazu wurden Mesokosmosexperimente durchgeführt, bei welchen Sedimente von einem Gletscherfluss und einem Grundwasserfluss reziprok transplantiert und zusätzlich mit Nährstoffen angereichert wurden. Diese Experimente wurden ebenfalls während drei verschiedenen Jahreszeiten durchgeführt, um mögliche zeitliche Fluktuationen zu erfassen.

Die Resultate der drei Studien zeigen, dass die verschiedenen Einzugsgebiete unterschiedliche bakterielle Gemeinschaften und mit ihnen verknüpfte Funktionen aufweisen. Die Separation zwischen Grundwasser- und Gletschersystemen und die Stärke ihrer temporalen Dynamik zeigen in Bezug auf Struktur und Funktion unterschiedliche Ausprägungen in den einzelnen Einzugsgebieten. Die physikochemikalischen Eigenschaften diktieren die bakteriellen Strukturen und teilweise ihre Funktionen. Die Grundwassersysteme zeigen höhere zeitliche Stabilität und eine kleinere Kopplung zwischen Struktur und Funktion, was auf eine Dominanz von Generalisten in diesen Systemen hindeutet. Die bakterielle Gemeinschaftszusammensetzung innerhalb der Uferzone als auch im hyporheischen Sediment scheint von der hydrologischen Situation der Systeme, sprich dem landschaftlichen Vernetzungsgrad, und der vorherrschenden Strategie der anwesenden Bakterien abhängig zu sein. Des Weiteren zeigen die Resultate, dass hydrologische Prozesse einen starken Einfluss auf die bakterielle Funktionalität alpiner Überschwemmungslandschaften ausüben. Abschliessend zeigen die Resultate, dass die bakteriellen Gemeinschaften ein hohes Mass an Resistenz gegenüber Störungen, wie etwa veränderte Wasserressourcen oder erhöhte Nährstoffkonzentrationen,

aufweisen. Dies zeigt sich für die einheimischen Gletscher- und Grundwassergesellschaften, wobei die letzteren eine höhere Resistenz aufweisen. Erstaunlicherweise war eine ausgeprägte funktionelle Plastizität/Redundanz zu allen Zeitpunkten vorhanden, was zu einer raschen Anpassung an die neuen Umweltbedingungen führt.

Die präsentierten Resultate zeigen die hierarchisch strukturierten, komplexen und teilweise verknüpften Faktoren, welche bakterielle Gemeinschaften und ihre Funktionen beeinflussen. Diese Gemeinschaften und ihre zugehörigen Funktionen werden sich höchst wahrscheinlich in Übereinstimmung mit den voranschreitenden Gletscherrückzügen und Änderungen der ökohydrologischen Umwelt wandeln.

1 Introduction

1.1 Microbial ecology in alpine floodplains: Why?

Heterotrophic bacteria (eubacteria and Archaea) play an integral role in the functional ecology of ecosystems as they embody the main actors in metabolic processes such as respiration and productivity, nutrient cycling and fluxes, as the trophic link with secondary consumers, and being involved in numerous biogeochemical processes (Edwards *et al.,* 1990, Kirchman 1994, Naegeli and Uehlinger 1997, Hall and Meyer 1998, Acuna *et al.,* 2008, Böhlke *et al.,* 2009, Cooney and Simon 2009). There are estimates that earth houses up to 6 x 10^{30} microbial cells with an estimated diversity of 10^3 to 10^9 species(Schloss and Handelsman 2004). Their versatile abilities and the ubiquitous appearance of bacteria not only make them an interesting study objective, but in a world that faces increasing human induced alterations, an understanding of underlying mechanisms driving biodiversity and ecosystem services provided by bacteria becomes highly relevant.

Alpine regions play an important role at a global scale and represent isolated landscapes which experience relatively high environmental stress levels. Aquatic ecosystems in alpine environments are strongly influenced by cryospheric and hydrological processes (Milner *et al.,* 2009).These processes, which depend on atmospheric forcing and snowpack/glacier mass-balance, are interlinked to river discharge, physico-chemistry and ultimately the biota and their provided services. Alpine areas show high precipitation rates, thus providing large quantities of fresh water to lower elevation regions intensively used by humans. Landscape structure and climate within alpine catchments dictate hydrological characteristics. A large fraction of the precipitation is stored as snow and ice in glaciers during winter. In the warm seasons, this water is released as snowmelt and glacial-melt waters that feed floodplains. Typically, groundwater channels are found within these floodplains and contribute to the total water budget (Brown *et al.,* 2003, Pedrós-Alió 2006). Environmental conditions such as diel and annual discharge fluctuations, physico-chemical characteristics and hydrological connections are tightly linked to these channel types, thus driving landscape heterogeneity (Ward 1994, Brown and Fuge 1998, Smith *et al.,* 2001). Climate models predict increasing temperatures in the European Alpine areas with drastic consequences for the glacial mass balance and characteristic flow regime (Horton *et al.,* 2006, Zemp *et al.,* 2006, IPCC 2007).

Hydrology, hydrochemistry, channel expansion and retraction are expected to change substantially in concert with an alteration in climate. Glacial recession is likely to lead to an altered discharge and discharge pattern. For instance, on a shorter time scale there is likely to be an increased discharge due to increased glacial mass loss. This can substantially change water temperature, channel stability, suspended sediment concentrations but also the active channel zone (Milner *et al.,* 2009, Brown *et al.,* 2010). On a longer timescale, glacial water input will become mitigated or even disappear, thus the importance of ground water and snow melt will become more dominant. Vegetation can show elevation shifts due to higher temperatures, precipitation and CO_2 concentrations, ultimately leading to a different carbon budget within these systems (Theurillat and Guisan 2001). Seasonally fluctuating instream primary production and hydrologically mediated leaching from surrounding soils and vegetation can substantially furnish Alpine streams with organic matter that is eventually decomposed by the heterotrophic microbial community associated with surface biofilms and hyporheic sediments (Boyer *et al.,* 2000). Altered hydrologic connectivity between streams and between streams and the riparian zone may influence the relative input of terrestrial versus autochthonous organic matter (Hood *et al.,* 2005, Judd *et al.,* 2006). Atmospheric deposition substantially supplies alpine regions with nitrogen and is likely to move its seasonal peak input from melting snowpacks in spring to an earlier occurring and more constant perennial one (Burns 2003, Hiltbrunner *et al.,* 2005, Magnusson *et al.,* 2010). Weathering, glacial scouring of bed rock and hydrological interactions with the riparian zone provide phosphorous to aquatic systems (Tockner *et al.,* 1997, Bünemann *et al.,* 2011). With retreating glaciers and changing climatic characteristics, the nutritional state of alpine ecosystems will likely change in the near future.

Mountain regions house a wide variety of habitats with endemic organisms due to their insularity and thus are highly sensitive ecosystems and valuable indicators of environmental change (Battin *et al.,* 2004). Alpine systems will likely change their hydrologic characteristics with decreasing importance of glacial water and increasing importance of ground water and precipitation runoff (Uehlinger *et al.,* 2010). Shifts in water source, landscape heterogeneity and connectivity, seasonality, and nutritional state can influence habitat templates and thereby change bacterial community structure, dynamics and functioning (Judd *et al.,* 2006, Uehlinger *et al.,* 2010, Winemiller *et al.,* 2010). Despite the importance of glaciated alpine catchments for providing large quantities of water also to lower elevation landscapes, there is surprisingly little knowledge about the pivotal ecosystem services mediated by bacteria in these systems. This includes the characteristics of bacterial communities and their associated functions in time and space. We use the services of microbes in many areas of life such as biotechnology and engineering, food processing, soil

remediation and wastewater treatment. The inclusion of microbial mediated services at the landscape scale not directly related to agriculture into the assessment of landscape conditions and management to enhance these, still seems to be in its infancy. Ecosystem components were dominated by microbes for most of the last 4 billion years in life history. They controlled elemental cycling, organic matter production and turnover as well as the climate (Smil 2003, Ducklow 2008). Anthropogenic activities have become an integral and major ecosystem component within the past century. The disturbance of chemical composition of the atmosphere and the alteration of the nitrogen cycle with an increase of 100% of all reactive nitrogen entering terrestrial ecosystems are just a few (but important ones) to name (Vitousek *et al.,* 1997). Thus humans have become an important player in demanding and determine ecosystem services. Around 50% of all accessible surface freshwater is used by human activity whereof 70% are used for agriculture (Vitousek *et al.,* 1997). As microbes are essential for, i.e., biogeochemical processes and thus water quality, it becomes important to incorporate this supporting ecosystem service into a socio-ecological framework taking account both parties (Millenium Ecosystem Assessment, 2005). There is much potential to improve or design models that cover this aspect of microbial life and its interaction with humans. Mechanistic understanding of microbial community dynamics can contribute to more incorporated and practical applications such as ecosystem restoration or eco-engineering (Gerbersdorf *et al.,* 2009, Richardson *et al.,* 2011, Salazar *et al.,* 2011).

1.2 Microbial community assembly, related functions and their spatio-temporal dynamics

1.2.1 Microbial diversity

Microbial ecology is a scientific field that has a tremendous number of questions. One of the main interests therein is the understanding of what drives bacterial community assembly, related ecosystem functioning and their spatio-temporal variation, thus their biological diversity.

Biodiversity, as defined at the convention on biological diversity in 1992, is the variety among life forms of any origin within an ecosystem, biome or an entire planet (Hawksworth 1995). This definition is rather general and covers many aspects of biological diversity such as species richness or species evenness (Tuomisto 2010). Levels of diversity can go beyond the commonly used species or the higher taxonomic level interspecies diversity and reach from ecosystem- to genetic- to molecular diversity (Campbell 2003). Ecological studies addressing the biodiversity in the geographical context of species patterns have existed for a long time (i.e. Humboldt and Bonpland 1807). Biogeography is the science that aims to assess and understand the mechanisms behind the spatial distribution of biological diversity (e.g. β-diversity) (Lomolino et al., 2010). Most focus has been on plants and larger animals and the geographical patterns of microbial life still remains poorly understood and only recently gained interest (Wilson 1992b, Mayr 1998, Horner-Devine *et al.,* 2004, Nemergut *et al.,* 2011, Brockett *et al.,* 2012). Furthermore, it is not just the spatial patterns but also temporal fluctuations of biodiversity that can occur within ecosystems and thus supplementary drive biodiversity within and across sites (Olapade and Leff 2005, Bottomley *et al.,* 2006, Edwards *et al.,* 2006, Crump *et al.,* 2009).

1.2.2 Diverse mechanisms driving bacterial diversification

Different mechanisms can affect biodiversity in time and space. The metacommunity framework takes an interaction of local and regional processes into account to synthesize possible mechanisms forming bacterial communities (Leibold *et al.,* 2004, Ricklefs 2004). Therein, regional and local processes drive local compositions of communities, which themselves are part of regionally connected and potentially interacting communities (Wilson 1992a, Leibold *et al.,* 2004). Four main perspectives are part of the frame work:

Common non-biotic driving factors on a geographic scale are, e.g., altitude, temperature, precipitation and geology (Fierer and Jackson 2006, Zinger *et al.,* 2009, Angel *et al.,* 2010, Fortunato and Crump 2011, Brockett *et al.,* 2012). Other factors influencing bacterial communities in soils and aquatic environments are pH, water availability, UV radiation, quality and quantity of organic matter and nutrients, productivity and the complexity of food webs (i.e. top-down and bottom-up effects) (Posch *et al.,* 1999, Fisher *et al.,* 2000, Crump *et al.,* 2003, Pernthaler 2005, Fierer and Jackson 2006, Logue and Lindström 2008, and references therein, Santos *et al.,* 2011, Van Horn *et al.,* 2011, Brockett *et al.,* 2012). The famous quote of Baas Becking: “Everything is everywhere and the environment selects”, covers these above mentioned mechanisms and constitute the classical view of bacterial diversity and distribution in space based on no dispersal limitation (Baas-Becking 1934). Biotic interactions (inter- and intraspecific) also can influence biodiversity. This interactions were shown to occur throughout species to kingdom boundaries and influence biodiversity of

species as well as functional levels and can lead to ecological associations (Goffredi *et al.,* 2007, Miniaci *et al.,* 2007, Fierer *et al.,* 2008, Duc *et al.,* 2009, Singh *et al.,* 2009, Chaffron *et al.,* 2010). These determinants interact with one another (i.e. Singh *et al.,* 2009) and ultimately influence the biological and physico-chemical milieu, thus creating environmentally different local habitat patches that are occupied by species capable of migrating to a different habitat patch and with variable ability to cope with apparent environmental conditions. This niche diversification drives bacterial biodiversity at regional scales and is denoted as (I) species sorting within the metacommunity framework.

Direct regional effects on habitat patches can be mediated by dispersal (passive or active) of bacterial cells from surrounding regions which then fuel a habitat patch. A (II) mass effect occurs when bacterial cells can invade environmentally heterogeneous habitat patches at a frequency that is higher than their extinction rate. This invasive occupation of a niche can uncouple species assemblage mechanisms to some degree from species sorting and thus generate benefits for less competitive but rapidly dispersing species. Depending on species traits and the grade of homogeneity of an environment, other dispersal mediated effects could occur. For instance, if species face a trade-off in colonizing homogeneous habitat patches and competing against invaders, there will be (III) patch-dynamics effects. This means that good colonizers are bad competitors and vice versa. Diversity will be determined by dispersal and species interactions in a patch-dynamic metacommunity (Lindström and Langenheder 2011). When species show no differences in their competitiveness species distributions will be stochastic; this represents the (IV) neutral perspective on community assembly. No niche differentiation due to trade-offs concerning local mechanisms will be apparent. Communities will drift over time due to dispersal events and speciation.

These four paradigms coincide and contribute differently towards the resulting bacterial community composition and functionality over time and space. Thus the distinctions between regional and local processes are usually not straight forward. Origin and dispersal, extinction and speciation, historical geographic-, biologic- and climatic conditions as well as landscape structure may influence bacterial communities as regional processes that can be seen in the context of historical biogeography (Martiny *et al.,* 2006, Fierer 2008, Lomolino *et al.,* 2010). Regional processes (historic or contemporary) can have an indirect influence on local conditions. For instance, dissolved organic matter input into a stream from surrounding areas and its processing when transported downstream can change environmental conditions of a local habitat patch (Wong and Williams 2010). Another example would be landscape structuring, such as the position of a stream or a lake relative to a forest, which can influence nutrient inputs but also water retention time (Yannarell and Triplett 2005, Crump *et al.,* 2007). The interplay of direct (dispersal) and indirect (spatially mediated environmental conditions) regional effects can essentially change the impact of local processes and interactions, thus changing the impact of species sorting as it would be present in, e.g., an isolated habitat patch.

1.2.3 Bacterial species concept, distribution patterns and bacterial strategies: The crux of the matter

The underlying mechanisms driving bacterial diversity are often highly complex, elusive and interconnected. Furthermore, there is not just a gap in the mechanistic understanding driving bacterial community composition but also difficulties to get hold of the large structural and functional diversity apparent within this microscopic world. As bacteria have existed since >3.5 Ga on Earth, the given diversity is not surprising (Forney *et al.,* 2004, Pedrós-Alió 2006). Over 9000 microbial species have been described to date (http://www.bacterio.cict.fr/number.html) (Schloss and Handelsman 2004). So far, it is not clear how many species can be expected on a global scale and estimates depend on individual bacteriologists (Curtis *et al.,* 2006, Pedrós-Alió 2006). For eukaryotes, there exist several species definitions that can be used depending on the scope of eukaryotic organisms (Mayden 1997). The species concepts for prokaryotes are controversial and have several limitations such as the impracticable use of morphologies (Potter *et al.,* 1997). The lack of a precise species distinction in microbiology, together with horizontal (i.e. lateral gene transfer or homologous recombination) and vertical genetic exchange makes it difficult to apply a theory-based species concept. Nevertheless, the species level is where several disciplines such as population genetics, evolution, genomics and ecology cross over (Rosselló-Mora and Amann 2001, Achtman and Wagner 2008). Many studies have used operational taxonomic units (OTUs) based on i.e. 16S ribosomal RNA (rRNA) sequences to assess microbial diversity. The genetic resolution strongly depends on the used methods and may be inappropriate to bin microbes into species. It would be preferable to use whole-genome comparisons to define species, but to date available complete bacterial genome sequences (~2000, NCBI Microbial Genomes Resources) would very likely not cover the diversity apparent in an environmental sample (Achtman and Wagner 2008). Anyway, increasing and accelerating (re)sequencing of metagenomic and single cell amplification studies will increase available genome sequences in the near future (Achtman and Wagner 2008). Today, species definition relies on more practical definitions such as DNA-DNA-hybridization (>70%) and 16S rRNA

gene identity (>97%) (Rosselló-Mora and Amann 2001). The definition based on 97% 16S rRNA similarity bears an underestimation of bacterial species diversity as the range of DNA-DNA hybridization in case of >97% 16S rRNA identity can vary between <20% and 70% and in the case of DNA-DNA hybridization of >70% there is generally a >97% 16S rRNA identity (Pedrós-Alió 2006). Alternative methods for defining microbial species which are consistent with the DNA-DNA-hybridization were used recently to define microbial species (Stackebrandt *et al.,* 2002). Multilocus sequence analysis is based on phylogenetic analyses of multiple protein-coding core genes which could delineate the extent of microbial species (Hanage *et al.,* 2006). The use of average nucleotide identity of all orthologous genes in complete genome sequences of pairs of strains may also be used to define species (Konstantinidis *et al.,* 2006). Furthermore, bacterial phenotypes (i.e. functions) are not necessary reflected by i.e. 16S rRNA sequences and are thus not congruent with the species definition (Jaspers and Overmann 2004, Fenchel 2005). Some microbial functions such as nitrogen fixation, denitrification, and use of certain carbon substrates (e.g. chitin or cellulose) are not restricted to distinct phylogenetic groups, but are widespread throughout bacterial domains. Anyway, it has been shown that species identity and distinct community composition can influence chitin and cellulose degradation rates (Peter *et al.,* 2011). Functional genes sometimes seem to be more directly related with bacterial community assembly than with defined bacterial species, and thus should be incorporated into studies focusing on community dynamics (Burke *et al.,* 2011). Species definition and used methods to describe them should may be guided by a method-free species concept which is based on cohesive evolutionary force as proposed by Achtman and Wagner (2008). Therein species are metapopulation lineages which consist of sets of connected subpopulaitons that are maximally inclusive. The limits of a lineage is set by evolutionary cohesive forces (de Queiroz 2005). A lineage can be thought of as a metapopulaiton that extends through time, occupies an adaptive zone minimally different from that of any other lieneage in its range and evolves separately from all lineages outside its range (Achtman and Wagner 2008). Thus, a metapopulation lineage consists over time even if genetic divergence within and between populations varies due to microevolution. Distinct to other species concept, metapopulation lineages not need to be phenotypically different, monophyletic, reproductively isolated or ecologically divergent to be species. They just have to evolve self-contained from other lineages (Achtman and Wagner 2008). Thus the evolutionary fate is the criterion which defines a species. Several ecotype-based population-structure models could lead to cohesive evolutionary forces (Cohan and Perry 2007). A specific ecological niche inhabits related microbes (ecotype) whose genetic diversity can be altered by several mechanisms. For instance, selective pressure of fitter ecotype variants can reduce genetic diversity. Formerly geographically seperated and diverged genotype clusters may compete after reunification. Diversity can be slowed down or persist owing to genetic drift within or homologous recombination between ecotypes. Lateral gene transfer may lead to the emergence of new ecotypes (Achtman and Wagner 2008). Regardless, besides the general attractiveness of the ecotype concept, such as evolutionary and ecological principles, several case-based biological observations indicate the need for adaptations (see Achtman and Wagner 2008).

The statement “Everything is everywhere, but the environment selects” squires microbial ecologysts whenever standing outside in the field (Beijerinck 1913, Baas-Becking 1934). As bacteria are small, produce resting stages, reproduce rapidly and potentially have high dispersal rates, it is very unlikely that they go extinct and thus they may be expected to show a cosmopolitan distribution (Fenchel and Finlay 2004). It is environmental factors at a specific place that dictates which species dominate a specific niche. Following this train of thought, there would be high local but a low global diversity and environmentaly similar niches would be dominated by the same species. The occasional-frequent model of Magurran and Henderson state mainly the same circumstance (Magurran and Henderson 2003). A set of “core” species represent the most frequent species in a community, as they can compete and proliferate best in a given environment. The “occasional” species represent a background community (I.e. Gobet *et al.,* 2012). Such a background can be maintained by seed banks or low frequency migration processes (Locey 2010, Lennon and Jones 2011, Lindström and Östman 2011). It is also possible that occasional species generally proliferate in a given environment but are surpressed by, e.g., viral lysis or protist predation (Pernthaler and Amann 2005, Sandaa *et al.,* 2009). Several studies indicate the existence of potential cosmopolitan bacterial species (Giovannoni *et al.,* 1990, Jezberová *et al.,* 2010, Chenal-Francisque *et al.,* 2011, Swan *et al.,* 2011), whereas others found biogeographical patterns, decreasing genetic similarity with distance or endemism (Cho and Tiedje 2000, Papke *et al.,* 2003, Whitaker *et al.,* 2003, Wawrik *et al.,* 2007, Peeters *et al.,* 2012). As molecular techniques were introduced in microbial ecology, most of the previously cultivated bacteria could not be detected (Pace *et al.,* 1986, Pedrós-Alió 2007). This suggested a large diversity but also revealed that some species may not be detected by these techniques. Nevertheless, if environmental conditions turn out to be appropriate, there is a chance that rare species will become more abundant or even take over (Pedrós-Alió 2007).

Moreover, there seem to be different strategies used in the microbial world as they are found for larger life forms (Pandit *et al.,* 2009, Vanschoenwinkel *et al.,* 2011). Generalist or specialist life history traits seem to be present in microbes (Fierer *et al.,* 2007b, Mou *et al.,* 2008). Dispersal mode and ability can differ between bacterial species (i.e. active motility vs. passive dispersal or

physical stress resistance) and make some species more likely to reach new habitats (Bissett *et al.,* 2010). The different strategies may also dictate which mechanisms primarily form and maintain bacterial communities. For instance, generalists would be more affected by patch dynamics and neutral processes, whereas specialists may be more influenced by species sorting and mass effects (Pandit *et al.,* 2009). Thus, species assemblages can have more then one mechanism depending on species traits, environmental conditions and landscape connectivity.

The problem of a consistent and clear species definition, the degree of connectivity between bacterial species and their functional capability (i.e. strategy, favored niche, etc.), the lack of knowledge of apparent biodiversity in a habitat patch and their spatial linkage (dispersal) makes microbial ecology a field in need of conceptual guidance. Positively said: There are myriads of questions left that scientist can dive into. Metagenomic analysis in different habitats have already shown a tremendous diversity in both microbial structural and functional diversity (Ram *et al.,* 2005, Yooseph *et al.,* 2007, Debroas *et al.,* 2009, Hewson *et al.,* 2009). Increasing accessibility to high throughput sequencing and emerging analytical tools will promote incorporation and junction of bacterial taxonomy and functionality into ecological concepts and reveal better understanding of the dynamics within the bacterial world.

1.2.4 Ecosystem functions mediated by microbes

The interrelationship of species diversity, available resources and possible ecosystem functions (i.e. productivity) of ecosystems is a long debated question. To date, there is no mechanism that explains why productivity-diversity relationships differ between communities, levels of biological organization and scales of observation (Cardinale *et al.,* 2009). The relationship between diversity and productivity differ, depending on the organism and the investigated scales (Waide *et al.,* 1999, Mittelbach *et al.,* 2001). For example, diversity can increase monotonically or as a concave-down function of productivity. The latter has been shown to occur often for plants at the local scale but can become a monotonically increasing relationship at regional scales (Chase and Leibold 2002). A reversed trend in this diversity-productivity relationship has been seen when diversity was decreased by experimental nutrient inputs (Gough *et al.,* 2000). Additionally, the hierarchy of linkages between biodiversity and ecosystem function is highly debated and it is not clear if biodiversity drives ecosystem productivity or if biodiversity is a consequence of productivity (i.e. the supply of resources) (Grime 1997, Huston 1997, Worm and Duffy 2003, Gross and Cardinale 2007).

Studies focusing on the relationship of bacterial community composition (richness and composition) and ecosystem function have revealed ambiguous results. Direct relationships between biodiversity, community composition and rates of ecosystem function such as metabolic activity have been reported (Bell *et al.,* 2005). Coupling between community structure and function can also be weak. Langenheder *et al*. (2006) showed that different bacterial founder communities can develop under standardized environmental conditions to still divergent assemblages but with same broad scale functions such as respiration and biomass production. Regardless, more specific microbial processes can often be related to a distinct phylotype and thus is not necessarily related to community richness (Langenheder *et al.,* 2006, Peter *et al.,* 2011).

When an ecosystem's environmental conditions show different levels of heterogeneity (i.e. different carbon resources) and biodiversity, there can be a general change in broad scale processes related to either of these two factors. More complex interactions with specific apparent species and distinct environmental parameters still can occur and influence the final outcome of a process (Langenheder *et al.,* 2010). In an experiment where different communities faced a set of environmental conditions, there was only a weak coupling to the functions as the community shifted (Langenheder *et al.,* 2005). Interestingly, the communities were more similar when they had the same inoculum or the same medium, indicating that populations were comprised of populations with the life history strategy of generalists and specialists. Accordingly, the linkage between microbial structure and function depends on the scope of processes that are investigated. Specific functions (i.e. specific enzyme activity) tend to be linked more tightly to community change than broader functions that are mediated by wide-spread metabolic capabilities within bacterial communities such as respiration (Langenheder *et al.,* 2006). Also in this area of research, the upcoming molecular possibilities will bring a better understanding of how broad and fine scale processes are linked to community composition, diversity and environmental factors.

1.2.5 Enzymes providing ecosystem functions

Studying enzyme activities as an ecosystem function is of great interest, as they provide insight into metabolic capabilities and activities. Furthermore, they can reflect environmental conditions and possible biologic interactions. Enzyme activities have been

shown to vary over time within an ecosystem, as they respond rapidly to changing environments. Seasonal variations are typical for enzyme activities and can be linked to different underlying processes like temperature alterations, water availability or litter inputs (Jones and Lock 1993, Romaní and Sabater 2001, Frossard *et al.,* 2012). Several mechanisms have been reported that are involved in enzyme activity variation. Factors related to chemical properties of growth substrates can regulate enzyme expressions, such as the quality and quantity of organic matter and the availability of nutrients (Findlay *et al.,* 1997, Findlay and Sinsabaugh 2003, Findlay *et al.,* 2003, Romaní *et al.,* 2004, Rulík and Spáčil 2004, Artigas *et al.,* 2008, Artigas *et al.,* 2009) or pH (Simon *et al.,* 2009). Also, physical and physico-chemical related mechanisms seem to have regulatory impacts on enzymatic activities. Structure of the streambed, grain size, discharge, temperature and water availability have been shown to affect enzyme activities (Battin 2000, Romaní and Sabater 2000b, Romaní and Marxsen 2002, Rulík and Spáčil 2004, Romaní *et al.,* 2006b, Zumsteg *et al.,* 2011, Brockett *et al.,* 2012). Direct and indirect biological interactions at large and small scales also can influence enzyme expressions (Miettinen *et al.,* 1996, Romaní and Sabater 1999, Romaní and Sabater 2000a, Romaní *et al.,* 2006a, Ylla *et al.,* 2009, Pohlon *et al.,* 2010, Van Horn *et al.,* 2011).

1.3 Aims and scope of the thesis

The main objective of the thesis was to gain a better understanding of mechanisms driving spatio-temporal dynamics in bacterial community composition and their related functions within stream sediments of glaciated alpine floodplains and gain insight into possible future changes within these ecosystems.

A set of potential underlying mechanisms driving bacterial community composition, and their mediated ecosystem services, were investigated during different seasons. They extended from large-scale factors such as geographic position and general environmental conditions (i.e. water source) to more small-scale factors such as hydrological mediated processes in sub-compartments of a floodplain. Thus, an array of local and regional factors was covered in a temporal context. Also, biotic aspects of how tightly linked and flexible are functions within bacterial communities and the communities themselves were of central interest within the thesis.

1.4 Study systems

The studies were conducted within three Alpine glaciated floodplains. They are situated in the Engadin and Wallis regions in Switzerland.

The Val Roseg catchment is situated in the eastern Swiss Alps and belongs to the lower austroalpine Bernina nappe (9°53’53’’E, 46°29’24’’N). The Roseg River is an 11.3-km long second-order tributary of the river Inn, which drains into the Danube. Approximately 30% of the water volume of the Roseg River is fed by water from two valley glaciers, the Roseg glacier and Tschierva glacier, both of which have retreated continuously over the last century. Permanent flowing first-order tributaries contribute groundwater and snowmelt to the Roseg River.

The Loetschental catchment is in the Rhone-Valley in the southwest part of the Swiss Alps (07°49'03"E, 46°25'08"N), harboring the second-order kryal stream Lonza which drains into the Rhone. The kryal tributary fed by the Jegi glacier and several first-order krenal tributaries drain into the Lonza within the study area.

The Macun Lakes region is a high alpine cirque situated in the Swiss National Park, and located in the mid-eastern part of the Swiss Alps (10°07'31"E, 46°43'51"N). The catchment is divided into a southern and northern basin that differs in their water source. The northern basin is mainly groundwater and snowmelt fed, whereas the southern basin is fed mostly by rock glaciers. Differently sized and connected lakes are present.

In all systems, most tributaries run dry during winter and the channel network experiences a contraction period.

For detailed information on the catchments, see appendix chapter 1.

1.5 Projects

In the first chapter, I conducted a seasonal sampling campaign within the three above mentioned floodplains to cover an array of landscape features. Sediment samples were taken during three periods that show distinct differences in hydrology and physico-chemical system characteristics. Groundwater and glacial water systems were chosen as two contrasting systems. The bacterial community structure and functioning were assessed by means of automated ribosomal intergenic spacer amplification (ARISA), catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) and the measurement of potential enzyme activities. I found each catchment to contain a distinct bacteria community structure and different degrees of separation in structure, functioning, and seasonality between stream types. Structural bacterial separation between stream types depended strongly on physico-chemical characteristics, whereas functions were just partially separated by water sources. Moreover, the magnitude of separation between stream types depended on the specific physico-chemical properties of the water within each catchment. Seasonality in structure and function was more apparent in glacial systems, whereas groundwater systems were more temporally stable. Finally, the linkage between structure and function was less apparent in krenal than kryal channels, indicating a greater prevalence of generalists in groundwater-fed streams.

The second chapter assessed the importance of hydrologically-mediated physico-chemical and biotic connectivity between floodplain habitats in driving bacterial community composition and ecosystem functions. Nutrients are transported along the flow path within a floodplain and can undergo transformation and cycling along this path. Additionally a passive bacterial dispersion can be mediated by hydrological connectivity and potentially have an influence on the community structure and/or their mediated functions within a habitat patch. I installed piezometers within the hyporheic and riparian zones in two alpine floodplains in Val Roseg differing in landscape structure, using them as incubation chambers for bacteria to investigate the impact of hydrology on bacterial community structure and function. One site was investigated during three different hydrological periods to include the potential effects of altered hydrologic connectivity. My results suggest a strong influence of hydrological connectivity on bacterial functioning (enzyme activity) corresponding with directed changes along each flow path. Community assembly (ARISA) was less influenced by the hydrologic linkage, indicating a high degree of functional plasticity within alpine bacterial communities. A distinction of hyporheic and riparian zone bacterial communities appeared dependent on the landscape structuring within each floodplain. An emerging importance of non-directed spatial processes (i.e. the impact of non-directed dispersal of microbes or non-gradual physico-chemical habitat conditions) drove bacterial assembly and function during hydrological less-active periods.

In chapter three, I was interested in the impact of altered water source and nutritional state that these ecosystems potentially face in the near future. Therefore, I conducted a reciprocal transplantation experiment of hyporheic sediments within the Val Roseg catchment in the same distinct three periods as in chapter one. Bacterial communities and functions were assessed after they were either relocated into the non-native water source, provided with an altered nutritional state, or both. ARISA and enzyme activities were mainly used as parameters showing bacterial response to this altered environment. Surprisingly, I found that bacterial community composition in both glacial and groundwater systems were highly resistant to the experimental disturbance and exhibited pronounced flexibility concerning their enzymatic activities. Major factors determining structure and function were the origin of sediments and seasonal variation. The native groundwater communities were generally more stable compared to the native glacial ones, although both showed the same extent of functional plasticity. Again, this difference in connectivity between community composition and ecosystem function indicates that groundwater communities are dominated by generalists, whereas glacial systems are dominated by specialists. I only detected a weak effect of altered nutrient supply on structure and function, which suggests a highly complex but hierarchically structured relationship of the tested factors; i.e., a historical and seasonal component that is dominant over the change in physico-chemical milieu, on the investigated time scale.

Paper 1

Spatio-Temporal Patterns and Associated Functions of

Lotic Bacterial Communities in Three Alpine Floodplains

Spatio-Temporal Patterns and Associated Functions of Lotic Bacterial Communities in Three Alpine Floodplains

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Abstract

Glaciated alpine floodplains are responding quickly to climate change through shrinking ice masses. Given the rapid changes in their physico-chemical environment, we anticipated highly variable structure, functionality and spatio-temporal patterns in hyporheic microbial communities of pro-glacial alpine stream sediments. We examined microbial structure and functioning during different seasons in glacial (kryal) streams and, as contrasting systems, groundwater-fed (krenal) streams. Three catchments were chosen to cover an array of landscape features such as interconnected lakes and local geology. Community structure was assessed by automated ribosomal intergenic spacer amplification (ARISA) and catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH) and microbial function by potential enzyme activities. We found each catchment to contain a distinct bacterial community structure and different degrees of separation in structure, functioning, and temporal shifts between stream types. For instance, we found a strong influence of stream type on structure but only partial separation of bacterial functioning. The magnitude of separation between stream types was dependent on the physico-chemical properties of the water within each catchment. Temporal shifts in structure and function was more apparent in kryal systems, whereas krenal systems were more temporally stable. The linkage between structure and function was less apparent in krenal than kryal channels, indicating a greater prevalence of generalists in groundwater-fed streams. With the rapid retreat of glaciers and therefore altered ecohydrological characteristics, lotic microbial structure and functioning are likely to change substantially in pro-glacial floodplains in the future. This change should be taken in account in future models predicting ecosystem services of alpine landscapes.

Introduction

Heterotrophic bacteria are crucial in the functional ecology of aquatic ecosystems, being the driving force behind metabolic processes like respiration and productivity, nutrient cycling and fluxes, trophic links with secondary consumers and numerous biogeochemical processes (Edwards *et al.,* 1990, Kirchman 1994, Naegeli and Uehlinger 1997, Hall and Meyer 1998, Acuna *et al.,* 2008, Böhlke *et al.,* 2009, Cooney and Simon 2009). The hyporheic zone and its heterotrophic components play an important role by integrating many of these ecosystem functions at the interface between surface waters, groundwaters and the riparian zone (Hendricks 1993, Stanford and Ward 1993, Findlay 1995, Battin 1999). These aquatic systems are undergoing rapid change in response to glacier recession, thereby providing the opportunity to examine responsiveness in bacterial community structure and function (see Milner *et al.,* 2009) while also providing a description of novel environmental conditions in high elevation lotic systems influencing this responsiveness.

Globally, alpine catchments are major sources of freshwater due to relatively high levels of precipitation, often stored as snow and ice in glaciers. This stored water is then released during warm periods as snow and glacial-melt waters. Groundwater-fed streams also are common in alpine catchments. Hence, the majority of running waters in glaciated alpine floodplains consist of glacier-meltwater-fed (kryal) and groundwater-fed (krenal) channels, and streams dominated by snowmelt (rhithral) during spring (Brown *et al.,* 2003). These different types of streams in Alpine landscapes have distinct annual and diel discharge patterns (flow regimes), hydrological linkages and physico-chemical characteristics (Ward 1994, Tockner *et al.,* 1997, Brown and Fuge 1998, Smith *et al.,* 2001). Krenal systems, for example, are less influenced by discharge fluctuations, whereas kryal systems show high discharge during summer ablation and an increasing influence of groundwater towards winter (Brown and Fuge 1998).

Regional climate models predict an increase in mean temperature in European Alpine regions and a more pronounced negative glacial mass balance (Horton *et al.,* 2006, Zemp *et al.,* 2006, IPCC 2007). Krenal systems will likely become more common as glaciers retreat and precipitation patterns change; e.g., projections suggest that precipitation periods will shift from reduced precipitation in summer towards increased precipitation in late winter (Swiss Climate Change Scenarios CH2011). Landscape heterogeneity, as influenced by glaciers, will be reduced and a consequent shift in flow regime towards more krenal-regulated systems is expected. This shift in water source will have a large effect on the physico-chemical and ecological state of alpine lotic systems (Fagre *et al.,* 1997, Ebert 2002, Hall and Fagre 2003). For instance, the quality, quantity and timing of resources, such as organic matter and nutrient inputs, are highly affected by shifts in environmental and hydrological conditions, and will likely influence heterotrophic bacteria assemblages and their ecological services or functioning (Boyer *et al.,* 1997, Findlay and Sinsabaugh 1999, Tockner *et al.,* 2002, Sobczak *et al.,* 2003, Horton *et al.,* 2006).

To date, there is no clear general understanding of biogeographical, environmental and temporal patterns in, and causes for, bacterial assemblage structure or function among freshwater habitats (Fenchel 2003, Dolan 2005, Lindström *et al.,* 2006, Van der Gucht *et al.,* 2007, Logue *et al.,* 2011). In this study, our main objective was to assess the spatio-temporal dynamics in bacterial community composition (BCC) and enzymatic functioning (EF) within hyporheic sediments in glaciated alpine floodplains in relation to potential environmental drivers. We examined kryal and krenal streams within three different alpine catchments, focusing on spatial and temporal differences in physical-chemical characteristics of these streams. Because of the more pronounced physico-chemical and temporal heterogeneity within kryal systems, we hypothesized that microbial communities will show stronger structural and functional temporal patterns and generally more spatial heterogeneity, whereas krenal systems would show more homogeneous patterns. An additional objective was to anticipate potential future shifts in bacterial communities and their ecosystem functioning within alpine catchments in relation to expected global changes in alpine water regimes. Three catchments were chosen due to their relative differences in deglaciation, geological background, and stream network structure, thus providing more generality to the findings.

Material and methods

Study floodplains

Geographical location, geological characterization and hydrological conditions in the three study sites, Val Roseg (VR), Loetschental (L) and Macun (M) are given in Table 1 (sources: Labhart 1998, Malard *et al.,* 1999b, Robinson and Kawecka 2005, Schmidt *et al.,* 2009, BAFU 2010).

The water volume of the Roseg and Loetschental catchments are partly fed by water from valley glaciers, which have retreated continuously over the last century (Maisch 1988, Tockner *et al.,* 1997, Malard *et al.,* 2000a, Tockner *et al.,* 2002). Permanent flowing first-order tributaries contribute groundwater and snowmelt to the kryal main channels, which have peak flows during spring and summer (Malard *et al.,* 2000a). Loetschental study sites also are divided between two sub-catchments (Figure 1). The Macun Lakes region is a high alpine cirque. The catchment is divided into a southern and northern basin that differs in their water source (Robinson and Matthaei 2007). The northern basin is mainly groundwater and snowmelt fed, whereas the southern basin is fed mostly by rock glaciers (Figure 1). All catchments experience contraction of surface channels in winter (Robinson and Matthaei 2007).

Sediment sampling

Hyporheic sediment samples for bacteria were collected from selected sites in all three catchments during summer (A) and fall/winter (O) 2008 (i.e., July/August and October). The Val Roseg and Loetschental catchments were also sampled in spring (J) June 2009. A total of 118 samples were collected: 10 kryal sites and 8 krenal sites in the Val Roseg (VR1-VR18), 4 kryal sites and 6 krenal sites in Loetschental (L1-L10), and 6 kryal sites and 11 krenal sites in the Macun catchment (M1-M17) (Figure 1). Water systems were distinguished based on previous studies (Tockner *et al.,* 1997, Robinson *et al.,* 2007) or determined by geographical position relative to the glaciers. Thirteen sites were not included in the data analysis: 5 sites that were dry in Val Roseg and Loetschental in winter (VR3, VR17, L2, L3 and L8), site VR11 spring sample due to a potential contamination, and Macun samples M1, M5, M11, M13, M15, M16, M17 that were snow-covered during winter and therefore not accessible.

For each sediment sample, the upper ~10 cm of streambed sediment was removed. Sediment samples were then taken to a depth of approximately 20 cm, sieved through an 8-mm mesh sieve (Retsch GmbH, Germany). All samples were transported in a cooling box to the laboratory, where samples for DNA extraction and enzyme assays were frozen at -20°C. Fixation for microscopic analysis was performed within 12 hours of sampling (see below).

Physico-chemical water parameters

Specific conductance (µS cm^{-1} at 20°C) and temperature were measured in the field with a conductivity meter (LF323, WTW, Weilheim, Germany). Surface water samples (1L) were collected and transported in a cooling box to the laboratory. The water was then filtered through pre-ashed glass fiber filters (GF/F, Whatmann) and the filtrate analyzed for dissolved organic matter (DOC), particulate organic carbon (POC), total inorganic carbon (TIC), ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), dissolved organic nitrogen (DON), particulate organic nitrogen (PN), phosphate (PO_4-P), dissolved phosphorus (DP) and particulate phosphorus (PP) according to standard protocols detailed in Tockner et al. (1997). We chose to sample surface water instead of pore water as most sites showed relatively coarse substrate (see sediment sorting coefficients below), thus a strong similarity of surface and subsurface waters within the upper sediment layers is likely.

Sediment characteristics

Sub-samples of the collected sediments were air-dried at 50°C and then used to measure pH as described in Schofield and Taylor (1955). Total sediment organic matter (OM) was determined as ash free dry mass (AFDM) by combusting the samples at 450°C for 4 h. The remaining material was then used to assess the grain size distribution using a sieving machine (Retsch GmbH, Germany) with mesh sizes of 6.3, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.063 mm. The size distribution was analyzed using GRADISTAT software (Simon J. Blott 2001) to determine the D90/D10 sorting coefficient as a measure of sediment interpacking.

Bacterial total cell numbers and CARD-FISH

A 0.5 ml aliquot of collected sediment was suspended in 1.11 ml paraformaldehyde (2%, final concentration) in an Eppendorf tube and fixed for 24 h at 4°C followed by three washing steps with 1 x PBS and 5 min centrifugation at 10,000 g between washing steps. Samples were then stored at -20°C in a 1:1 mix of PBS/ethanol until further processing (Pernthaler *et al.,* 2001). Cell detachment was done by sonication (Branson Digital Sonifier 250, Danbury, USA, 5-mm tapered microtip, actual output of 20 W, 30 s). The homogenate served as a template for filtration based counting of 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich Co) stained cells (Porter and Feig 1980) and specific phylogenetic staining with catalyzed reporter deposition fluorescence in-situ hybridization (CARD-FISH). Photographs for total bacterial counts were taken with an epifluorescence microscope (Leica Microsystem, DMI6000b) and analyzed with the CellC software (Selinummi *et al.,* 2005) or counted manually in case of high background fluorescence. CARD-FISH was performed following the protocol of Pernthaler *et al.* (2004) paired with a high throughput imaging system (Zeder and Pernthaler 2009). Horseradish labeled FISH probes (Biomers Inc, Ulm) EUB I-III targeting the domain *Bacteria* (Daims *et al.,* 1999), Alf968 and Bet42a affiliated with alpha and beta subclass of *Proteobacteria*, respectively (Manz *et al.,* 1992, Neef *et al.,* 1997), and CF319a assigned to the *Cytophaga-Flavobacteria* within the phylum *Bacteroidetes* (Manz *et al.,* 1996) were used to quantify microbial taxa within the stream sediments. Detailed information for this and the following sections of material and methods, statistical analysis and an in depth discussion on specific results (e.g. single chemical variables) are available in the appendix.

Enzyme assays

Eight different enzymes were tested for their activity using Methylumbelliferone (MUF)-labeled substrate analogues. They were chosen based on their potential role in bacterial metabolism. (Table 2, Vihinen and Mäntsälä 1989, Sinsabaugh *et al.,* 1991, Arpigny and Jaeger 1999, Makoi and Ndakidemi 2008, Sinsabaugh *et al.,* 2008). Fluorometric enzyme assays were performed as described before under standardized conditions (Findlay *et al.,* 2001).All values were corrected for quenching and potential auto fluorescence, i.e. due to presence of small mineral particles, and subsequently standardized to OM.

Bacterial community fingerprinting

BCC was assessed by automated ribosomal intergenic spacer amplification (ARISA). Samples were extracted using the PowerSoil DNA isolation Kit (MoBio, Carlsbad) following manufacturer's instructions. DNA was amplified using the fluorescein (6-FAM) labeled universal forward primer 1406f-6FAM and the bacteria specific reverse primer 23Sr (Yannarell *et al.,* 2003). ARISA fragment analysis was performed as described in Bürgmann *et al.* (2011).

Data analysis

All analyses were done using the vegan, relaimpo, stats and mgcv packages in R (R Development Core Team, 2011, Oksanen *et al.,* 2011).

Comparisons of environmental variables and cell abundance between catchments, water source and season were done using three-factor analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test. A suitable data transformation was performed where data did not meet normality criteria.

Community fingerprinting results and enzymatic activities were analyzed visually by combining them and the environmental data using NMDS with vector and factor fitting (biplots and dispersion ellipses). Permutational multivariate analysis of variance (PERMANOVA) was used to assess the influence of water source, catchment and season on community and enzyme activity structure with a full factorial model and to reveal potential linkages between them by means of pairwise comparisons (Anderson 2001). This assessment was supported by a Mantel test and a procrustes analysis of the NMDS ordinations (Mantel 1967, Gower 1975, Digby and Kempton 1987, Jackson 1995, Peres-Neto and Jackson 2001).

Correlations of single enzymes to physico-chemical factors were tested by multiple linear regression. Enzyme activities and environmental parameters were ln(x+1) transformed and OM was omitted as an independent variable prior to analysis to prevent autocorrelation. The models were selected with the Akaike information criterion (AIC). Significance of predictors were tested by

permutational ANOVA and their relative importance in the linear model was assessed using the lmg metric (Chevan and Sutherland 1991, Grömping 2006). Correlations of single physico-chemical factors on EF and BCC patterns were assessed by vector and factor fitting in addition with generalized additive models (GAMs) of environmental parameters fitted on the respective NMDS patterns (Bennion *et al.,* 2011, Oksanen *et al.,* 2011). Additionally, forward selected RDA models were built to assess the global importance of environmental parameters on BCC and EF. RDA based variation partitioning on Hellinger transformed ARISA and enzyme activity data was performed to evaluate the influence of chemical and physical (temperature and D90D10) factors on BCC. Unique fractions of RDA were tested by ANOVA-like permutation test (Peres-Neto *et al.,* 2006, Blanchet *et al.,* 2008a).

Multivariate homogeneity of group dispersion (MHGD), as an analogue of Levene's test for homogeneity of variance, was performed to assess beta diversity of enzyme activities, fingerprinting profiles and physico-chemical characteristics (Anderson *et al.,* 2006). Generally, multivariate dispersion (variance) was calculated by the distances of group members to a group centroid in multivariate space. Differences in dispersions can then be tested by permutational ANOVA. Lastly, Shannon diversity index was calculated for OTU's (operational taxonomic units).

Results

Physico-chemical characteristics

The catchments differed significantly in many physico-chemical parameters, e.g. temperature, sediment pH, and OM, as revealed by ANOVA, NMDS and Kruskal-Wallis tests (see discussion in the supplementary material and appendix Table 1). Significant differences were also found between krenal and kryal systems, e.g. for temperature, conductivity, pH and other parameters, but differences were frequently significant only for specific sites or dependent on season or catchment (significant interaction terms in ANOVAs). There were generally higher temperatures in the krenal systems. Macun sites had high OM, DOC, POC and PN concentrations and had lowest conductivity with no differences between the two water systems. DP was generally lower in krenal systems. PP was only different between the water systems in Val Roseg with higher concentrations in the kryal sites. Sediment pH was different between all catchments with lowest measures in Macun and highest in Val Roseg. TIC was higher in krenal systems and was lowest in Macun. NH_4 and NO_2 were different in Val Roseg between the two water sources with lower values in the krenal sites.

Ordination of environmental variables revealed differences in physico-chemical characteristics between water sources, catchments and season (PERMANOVA: $F_{1,89}$=21.22, $F_{2,89}$=24.59 and $F_{2,89}$=8.89, respectively, P<0.001, appendix Figure 1). The MHGD analysis showed an interaction of water source, season and catchment on beta diversity of physico-chemical characteristics (Permutation test: $F_{15,89}$=4.59, P<0.001, Figure 2). This result shows the differences in chemical heterogeneity of the systems within the different catchments during different seasons. An interaction of physico-chemical parameters between catchment and season, and catchment and water source was also apparent, indicating different strength in temporal fluctuations of physico-chemical parameters and separation of the two water systems in the different catchments (PERMANOVA: $F_{3,89}$=5.04, and $F_{2,89}$=8.52, P<0.001, appendix Figure 1).

Cell abundance and CARD-FISH

Bacteria cell abundance in sediments ranged from $1.66x10^6$ to $4.44x10^9$ (mean: $2.53 x10^8 \pm 6.75x10^8$, n=105) cells per g sediment dry weight (dw) and differed between the three catchments (ANOVA: $F_{2,89}$=71.45, P<0.001). Loetschental had the lowest mean cell densities (range: $2.65 x10^6$ to $1.90x10^8$ cells g^{-1} dw, mean: $3.47 x10^7 \pm 4.82 x10^7$ cells g^{-1} dw, n=27) followed by Val Roseg (range $1.67x10^6$ to $2.75x10^9$ cells g^{-1} dw, mean: $1.36 x10^8 \pm 4.02 x10^8$cells g^{-1} dw, n=51) and then Macun (range: $5.25 x10^7$ to $4.44x10^9$ cells g^{-1} dw, mean: $6.92x10^8 \pm 1.11 x10^9$ cells g^{-1} dw, n=27). There was a significant interaction between water source and catchment, with lower cell abundances in kryal sediments in Loetschental and Val Roseg (ANOVA: $F_{2,89}$=14.45, P<0.001, Tukey's HSD: P<0.001, appendix Table 1).

Hybridization rate for the EUBI-III probe ranged from 16.42 to 84.77% of all DAPI positive particles with a mean hybridization rate of 59.06±14.87%. Loetschental (range: 40.32 to 83.94% mean: 64.88±11.43%) and Val Roseg (range: 42.08 to 84.77% mean: 64.02±10.35%) had a higher mean hybridization rate than Macun (range: 16.42 to 65.97% mean: 43.84±14.98%, ANOVA: $F_{2,89}$=33.86, P<0.001, Tukey's HSD: P<0.001). The wide range could be due to seasonal and local presence

of Archaea. In winter there was s higher hybridization rate compared to summer (ANOVA: $F_{2,89}$=6.83, P<0.01, Tukey's HSD: P<0.01). There was a significant interaction between water, catchment and season with the EUBI-III hybridization rate mainly due to several differences in the low hybridization rates in the Macun catchment (ANOVA: $F_{3,89}$=3.23, P<0.05).

Alpha- and beta-proteobacteria were the most abundant groups according to CARD-FISH, and alpha-proteobacteria showed a strong distinction between season and catchment (ANOVA: $F_{2,89}$=7.7.74 and $F_{2,89}$=25.60, P<0.001). Beta-proteobacteria, had on average higher abundance in kryal sediments (ANOVA: $F_{1,89}$=9.65, P<0.01, Tukey's HSD: P<0.001) and were generally less abundant in summer in all catchments (ANOVA: $F_{2,89}$=4.05, P<0.05, Tukey's HSD: P<0.05). *Bacteroidetes* were least abundant with a range between 0.2 to 13.58% of all DAPI positive particles (mean = 3.06±3.08%).

Enzymatic activities

The patterns of enzyme activities varied considerably among sites. ANOVA revealed various significant differences between catchments and water source (Appendix Table 2 and 3). Seasonal effects were generally less pronounced.

The total activity of the enzyme set showed a difference between catchments and water source (ANOVA: $F_{2,89}$=38.06, P<0.001, $F_{1,89}$=6.32, P<0.05, respectively). A post-hoc test revealed generally highest mean enzyme expressions in krenal systems (Tukey's HSD: P<0.05) and highest mean values in Roseg, intermediate values in Loetschental and lowest values in Macun (Tukey's HSD: P<0.01).

MHGD showed that there was an interaction between water source, season and catchment in beta diversity of enzymatic activities (Permutation test: $F_{15,89}$=7.13, P<0.001, Figure 2). Diversity was high during spring in the kryal system in Loetschental and Val Roseg and in summer in Loetschental kryal systems. Krenal systems in Loetschental and Val Roseg have less seasonally driven diversification of enzymatic activities, whereas Macun had a stable enzymatic diversity during the sampled seasons in both water systems.

Ordination of EF showed strong separation between the two water systems, catchments and their interactions (Figure 3, appendix Figure 2), (PERMANOVA: $F_{1,89=}$40.51, P<0.001, $F_{2,89}$=18.92, P<0.001 and $F_{2,89}$=5.11, P<0.01, respectively) with clear separation of the three catchments and separation of kryal from krenal in Val Roseg and Loetschental. A seasonal shift in EF was partially present in Val Roseg in the pairwise comparison model, although not or only marginally significant in the complete model (PERMANOVA: $F_{2,89}$=2.02, P=0.08, appendix Table 4). Macun showed no functional separation between water sources (Figure 3, appendix Table 4).

Results of multiple linear regression show relationships of single enzymes with water chemistry, sediment characteristics and some of the nutrient availability variables (PP and pH most strongly). They are summarized in Table 3 and in the appendix Table 5. The EF patterns as a whole showed correlations with 11 of the 17 physico-chemical parameters when GAMs were fitted (appendix Table 6 and appendix Figures 4a to 4c). The fitted biplot vectors showed well the relative physico-chemical parameters potentially driving the functional separation between the two water systems (Figure 3). The RDA analysis accounted 50.4% of variation in EF to physico-chemical factors (physico-chemical, R^2_{adj}=0.504, $F_{5,99}$=22.15, P<0.001). Variation partitioning of separated water sources revealed for both an equally high contribution of chemical factors (kryal: 40.6%, krenal: 42.3%), whereas pure physical factors did not show a significant influence on EF or were intercorrelated with the chemical factors (see appendix).

Bacterial community structure and linked functions

A total of 191 OTU's were detected across all sites. Some 10 OTUs were unique to a single site, whereas 95 OTUs occurred in more than 50% of all sampled sites. Diversity (Shannon index) was highest in krenal systems (ANOVA: $F_{1,89}$=24.19, P<0.001). Beta diversity, as tested by MHGD, showed a significant interaction between water source, season and catchment (Permutation test: $F_{15,89}$=3.53, P<0.001, Figure 2).

NMDS ordination showed a differentiation in BCC between the two water sources and the three catchments, but a less pronounced seasonal effect (PERMANOVA: $F_{1,89}$=13.21, P<0.001, $F_{2,89}$=6.36, P<0.001, and $F_{2,89}$=1.57, P<0.05, respectively, Figure 4). There was also an interaction between water source and catchment (PERMANOVA: $F_{2,89}$=4.5, P<0.001). Krenal BCC were similar

in Loetschental and Val Roseg in summer and spring (PERMANOVA: $F_{1,13}$=0.90, P=0.709, $F_{1,13}$=1.07, P=0.378, respectively). Otherwise, there was always a significant catchment difference in BCC for the same water source within the same season (PERMANOVAs: P<0.05, appendix Table 4). Macun was more separated from the other two catchments in the NMDS, and showed a less pronounced separation of kryal and krenal sites, although they still were significantly different (PERMANOVA: $F_{1,26}$=2.09, P<0.01, Figure 4). Krenal systems showed no seasonal pattern compared to the temporal shift in BCC in kryal sites in Val Roseg (PERMANOVA: $F_{2,21}$=0.64, P=0.96, $F_{2,28}$=2.39, P<0.001, respectively). Seasonal BCC changes were not significant in Loetschental krenal and kryal sites (PERMANOVA: $F_{2,14}$=0.70, P=0.896, $F_{2,11}$=0.82, P=0.610, respectively). Macun BCC showed no seasonal pattern in kryal sites but did in krenal sites (PERMANOVA: $F_{1,8}$=0.77, P=0.791, $F_{1,16}$=1.75, P<0.05, respectively).

Fitting of the environmental data (Biplot vector fitting and GAMs) on the community NMDS showed that chemical factors correlating with the BCC differed between kryal vs. krenal systems with strength in gradients directing more towards the kryal systems. Particularly, gradients of sediment pH seemed to be of high importance for driving the community composition of the kryal systems of Loetschental and Val Roseg (Figure 4, appendix Table 7). The gradients of PP, DP, PO_4-P, DN, NH_4-N and NO_2-N towards the kryal sites of Val Roseg and Loetschental in summer mainly shows importance of glacial ablation on these factors and potentially on BCC. Temperature and OM seemed to correlate with krenal community composition (Figure 4, appendix Table 7 and appendix Figures 5a to 5c). RDA revealed that 19.5% of total variation was explained by forward selected environmental factors, which consisted of temperature, conductivity, pH, sorting coefficient, OM, POC, NO_3-N, NO_2-N and PP (physico-chemical: R^2_{adj}= 0.195, $F_{9,95}$=3.80, P<0.001, see appendix Table 7). Variation partitioning of physical and chemical parameters applied to the kryal systems showed 20.7% of the variation in community structure was accounted for solely by water chemistry. Krenal systems, in contrast, had just 9.3% of the variation explained by water chemistry. Physical factors were of minor importance in both systems (1.4% each). This result supports the trend in the ordination implying a higher influence of chemical conditions on BCC in kryal systems, although physical conditions also play an important role due to high intercorrelation with chemical parameters (see appendix additional information on enzyme activity). Krenal systems, in contrast, did not show a strong correlation with either chemical or physical factors (or their interaction), thus reflecting the more stable environmental conditions of krenal systems.

Relating enzyme activity patterns to BCC by means of pairwise comparison of PERMANOVA's (appendix Table 4) revealed a link between changes in assemblage structure and function in 63.3% of all pairwise comparisons. In Macun, a low 16.7% of all pairwise comparisons being significant indicated a weak linkage between structure and function. Roseg and Loetschental had linkages in 80% and 66.7% of all cases, respectively. Procrustes analysis of community and function NMDS resulted in a similar trend in the linkage between BCC and EF (r=0.639, p<0.001) when performed with all catchments included. Correlation of single catchments in the same ordination showed differences in strength of association with maximum correlations for Val Roseg, intermediate correlation for Loetschental, and minimal correlation for Macun (Table 4). The Mantel test also showed this trend: Correlation of structure and function of all catchments was 0.363 (P<0.01). The single catchments showed correlations of 0.561 (P<0.01) in Val Roseg, 0.389 (P<0.01) in Loetschental, and -0.102 (P=0.79) in Macun. For detailed procrustes correlations, also with environmental variables and split into distinct water systems, see table 4.

Discussion

Our results suggest a strong influence of water source on BCC, EF and spatio-temporal dynamics of bacterial assemblages within the hyporheic sediments of streams in glaciated alpine floodplains. Although the study floodplains showed distinct patterns in BCC, EF and temporal dynamics, water source primarily influenced heterotrophic bacteria occurrence, functioning, or both in all floodplains. Temporal shifts in BCC and EF were mainly evident in kryal systems, potentially mirroring their higher temporal heterogeneity in physical and chemical characteristics. Although temporal dynamics in BCC were not as apparent within krenal sites, there still was a remarkable difference in BCC and EF between catchments.

Hierarchical habitat templates in space and time

The patterns we observed appeared to follow a hierarchical template: e.g. global (geological) differences such as sediment pH or conductivity appeared to have a strong influence and act as a principal separator of bacterial community structure and enzymatic expression patterns between catchments. Fierer and Jackson (2006) compared soil samples across North and South America and found that soil pH explained community composition and richness. Landscape features such as glaciers have the potential to create strong landscape heterogeneity by dictating and fluctuating coarse-scale physico-chemical characteristics of

habitats over time and space. In contrast, upstream lakes or groundwater fed streams provide a more stable spatio-temporal and homogeneous landscape (Tockner *et al.,* 1997, Brown *et al.,* 2003).

The interactions of these global and regional factors form the habitat template for BCC and EF among alpine catchments and stream types (sensu Poff 1997). Temporal shifts in BCC and partly in EF in kryal channels are linked to shifting physico-chemical templates. The relatively large fraction of variance explained by physico-chemical factors as seen in the variation partitioning within the kryal system supports this finding. Thus, differential glacial inputs can be viewed as another principal separator of BCC and EF within and between catchments. In general, there was no or little temporal variation in bacteria function or structure in krenal streams. Variation partitioning only explained a small part of BCC dynamics in krenal systems via the influence of chemical and physical factors or their interactions. Regardless, EF was influenced to the same extent in kryal as in krenal systems, indicating large functional plasticity within this system.

Glaciers, lakes and snow: Landscape features that drive hydrology and bacterial communities

Seasonal glacial melt-water dynamics have a major influence on a suite of floodplain characteristics and therefore play a strong role in the ecology of alpine streams. For instance, bacterial communities within stream sediments appear to be strongly influenced by these temporal changes in environmental conditions, both physical and chemical.

Summer ablation leads to distinct physico-chemical water characteristics, increased sediment load, decreased channel stability and a greater extent in the hydrological linkage between aquatic and terrestrial compartments (Milner 1994, Ward 1994, Tockner *et al.,* 1997, Brown *et al.,* 2003, Battin *et al.,* 2004). We found that a suite of environmental variables can potentially influence BCC. Most of the physico-chemical variables show lower values during the ablation period and beta diversity of Val Roseg and Loetschental physico-chemical structure was relatively low. PP concentration was, on the other hand, highest in the Val Roseg catchment and was an important potential driver for a whole set of enzymes and thus may equalize enzymatic activity patterns over a large spatial scale. Accordingly, the large beta diversity in EF in Loetschental could be driven by differences in PP concentrations between the two sub-catchments. Beta diversity of the community was increased in concert with a decreased EF diversity in Val Roseg, thus suggesting a simplification rather than a specialization in process performance. This increased diversity may be due to generally lower concentrations of environmental factors normally constraining these communities and an increased physically driven constraint on BCC. Indeed, abrasion, shear stress and flow heterogeneity have been shown to play an important role in benthic biofilm formation, maturation, persistence and cell detachment and are likely to change further downstream (Besemer *et al.,* 2007b, Besemer *et al.,* 2009a, Singer *et al.,* 2010). The larger structural diversity in these kryal systems and the observed differences in BCC (and EF in Val Roseg) during the ablation period, may additionally be supported due to the stochastic promotion of bacteria species that are able to cope and interact within this harsh environment.

The fact that Macun EF did not differ between water sources may be driven by mitigated glacial water input, essentially homogenizing the two water systems. Kryal streams in Macun had no affiliation in either BCC or EF with kryal streams in the other two catchments. Community characteristics, such as total cell abundance, clearly showed this difference in the Macun catchment from the other catchments. Furthermore, patterns in benthic biofilms having lower bacterial abundance in kryal channels, as occurring in Val Roseg and Loetschental, also were not present within this catchment(e.g. Battin *et al.,* 2004). Small concentration of i.e. PP in both water systems and the low pH may reduce the potential for bacterial communities within the Macun catchment to perform distinct EF. The small differentiation between the BCC rather seems to be linked to an interplay of or an unknown factor, as there is no clear potential physico-chemical factor separating the two systems within Macun (see appendix Figure 5) The interconnected lakes in Macun can provide a high input of algal exudates during the ablation period, which feed both kryal and krenal streams (Robinson and Matthaei 2007). As a defusing agent of physical disturbance, lakes can act as a sinks of glacial-induced high sediment loads, therefore releasing algae from light limitation (Hieber *et al.,* 2002). The elevated Nac activity (Figure 3, appendix Figure 1) gives a hint about the importance of algae in the Macun catchment. Chitin is a constituent of diatoms that dominate Macun streams and lakes (Kawecka and Robinson 2008, Durkin *et al.,* 2009). The relatively low total EF per biomass (~OM) could then be due to repression of enzymes by assimilable algal exudates (Hoppe *et al.,* 1988, Guenet *et al.,* 2010). The presence of lakes may thus partially offset the differences in BCC and EF between stream types in the Macun catchment as they were found in the other two catchments.

Beta diversity in the physico-chemical structure of Val Roseg sites was higher during winter in contrast to the previously observed pattern (Tockner *et al.,* 1997). This pattern was mainly driven by a decreasing longitudinal effect of glacial melt. Some characteristics of glacial water (e.g. temperature) are modified as a function of distance from the glacier terminus (Smith *et al.,* 2001). Loetschental showed a similar pattern, although driven by differences between the two sub-catchments with one potentially getting large inputs from groundwater fed tributaries (sites L9 and L10, appendix Figure 1). Macun, in contrast, had low physico-chemical beta diversity in winter compared to summer (Figure 2). Beta diversity of BCC decreased during winter in all three catchments, likely due to an emerging homogeneity and decrease in temperature and reduced discharge fluctuations (Figure 2, appendix Table 7). NO_2-N and DP as potential drivers constraining BCC was also decreased and may have contributed to an equalization of community structures (appendix Table 7). Enzymatic patterns and enzyme beta diversity did not significantly change in winter compared to the ablation period, showing more consistent functioning that is maintained in winter despite changes in physico-chemistry. During the sampling period, there still was an increased concentration in PP which may have led to this stable EF pattern. Furthermore, extracellular enzymes can persist after cell death or senescence, adding a “lag” to these response variables (Kiersztyn *et al.,* 2012). The lack of a temporal shift in bacterial enzyme activity compared to the shift in community structure implies redundancy in ecosystem functioning as composition changes in kryal sites. Langenheder *et al.* (2010) found functional redundancy at a very low bacterial richness level as might be expected in winter.

Snow melt contributes large quantities of water to the floodplain during spring and thus increases hydrologic linkages and ecosystem heterogeneity along an altitudinal gradient due to different timing of snowpack melting. Pulses of nutrients are released into the soils and streams and can drive EF. The increased EF beta diversity we saw during spring is likely to be partly driven by this event. Specifically, the increased beta diversity in EF and BCC in Val Roseg could be partially attributed to the increased heterogeneity of sites situated downstream from the confluence of the glacier stream with the lake outlet stream. Additionally, there was an effect of incoming water from krenal channels downstream that further enhanced heterogeneity in the kryal system. Melting snow packs were amplifying the lateral water input into the kryal system as seen by BCC and EF convergence towards krenal characteristics (i.e. first axis of NMDS in appendix Figures 2 and 3), pointing out the importance of local factors generating landscape heterogeneity on a seasonal basis.

Coupling of bacteria structure and function

The results showed that the coupling between bacteria structure and function can be stronger or weaker depending on environmental constraints that determine local community structure. For instance, the Val Roseg and Loetschental showed strong coupling of function and structure within kryal systems, further suggesting that bacteria were specialists and performed distinct ecological functions. The lower Shannon diversity index in kryal systems also indicates the unevenness within the community towards potential specialists. Loetschental kryal systems demonstrated the strongest correlation between the environment and bacteria structure and function, implying a set of adapted specialist conducting relatively taxon-specific ecosystem functions. The high proportion of significantly fitted OTU's with a gradient towards the Loetschental kryal streams supports this finding (see appendix Figure 2).

Implications of global change for alpine stream microbiomes

Glaciers continue to recede and the future loss of snow and ice will alter alpine ecosystems in fundamental ways. It is expected that there will be a strong decrease in glacial meltwater input to alpine floodplains, altering spatial and temporal runoff dynamics. In general, a shift towards a more groundwater-dominated landscape is likely to occur in the near future in most alpine areas. This change in water source also entails a shift in the physico-chemical and structural habitat template. For example, temperature in kryal channels will increase in the future (Milner *et al.,* 2009). Water temperature showed a good correlation with BCC and EF and, although temperature did not strongly influence a single enzyme, it still could influence the EF pattern as a whole (appendix Table 6 and 7 and appendix Figure 5a and 6a). A shift in BCC and EF towards a Macun like characteristic may partially be possible, although global factors and landscape features such as pH, conductivity are likely to hinder a complete shift towards a BCC or EF characteristic as found in the Macun catchment.

Regardless, EF could experience a significant change with reduced or lost kryal water inputs. For instance, decreased concentrations of PP, DP, PO_4-P, NH_4-N and NO_2-N could promote a functional shift towards krenal characteristics or lead to generally higher enzymatic expression rates. Specific enzymes that correlate well with physico-chemical characteristics of kryal

water, such as Est and End, could become less expressed, whereas Alph, Bet, Xyl, Nac, Leu and Phos would dominate EF. These enzymes are involved, i.e., in the degradation of easy degradable but also recalcitrant substrates and would be suitably expressed if vegetation changes in concert with the climate induced change in glacial water.

Physical changes in the habitat template such as increasing channel stability due to reduced discharge may favor generalists and reduce stochastic proliferations as apparent in kryal channels (Milner *et al.,* 2009). For instance, Füreder (2007) proposed an increasing importance of macroinvertebrate generalists within kryal systems due to reduced glacial runoff. Reduced PP, PO_4-P, NH_4-N and NO_2-N concentrations could favor generalists as well as elevated OM within the sediment (Figure 4, appendix Table 7 and Figure 5). OM can originate from benthic algal inputs that grow efficiently within the krenal channels. In-stream primary production could become more important in the future for kryal systems, thus fueling hyporheic sediments with OM and thus promoting a shift towards a krenal BCC characteristic (Logue *et al.,* 2004, Uehlinger *et al.,* 2010). Another parameter that is expected to increase is the total abundance of microbial cells. Although we did not see a decreased overall OTU occurrence in kryal systems, a future shift towards a more even community structure and loss of specialist species could be caused by decreased glacial runoff in kryal systems. This would lead to a reduced temporal and spatial species turnover, and decreased beta diversity at the landscape scale.

An altered physico-chemical habitat template is likely to influence EF in both water systems. As BCC are more stable within krenal systems while having a high functional plasticity, we would expect to see a less pronounced shift in community composition and a longer time horizon until changes appear. In contrast, kryal system BCCs could change more rapidly and pronounced as they seem to be more constrained by physico-chemical factors due to a potential reduced functional plasticity.

Our study shows how lotic microbial structure and function in Alpine floodplains will potentially change as a consequence of altered hydrological conditions. As microbes play an important role linking geochemical organic matter, nutrient cycling and higher trophic levels, a significant shift in alpine floodplain foodwebs and ecosystem function can be expected. However, so far it is not sure to what extent a shift of kryal BCC would be buffered if their provided EF could completely adapt to a changed physico-chemical environment guaranteeing a similar ecosystem functioning or if these communities will be gradually replaced.

Acknowledgements

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Titles and legends to figures

Figure 1

Map of the study catchments and location of sampling sites in (A) Val Roseg, (B) Macun, (C) Loetschental. Kryal sites have an asterisk. Streams are delineated in grey. Lakes and glaciers are depicted as dark grey and grey areas, respectively. Light grey indicates the sub-catchment in Loetschental and the moraine area in Val Roseg.

Figure 2

Boxplots of distances to group centroids on the first two PCoA axes as assessed by MHGD. Whiskers indicate 1.5x interquartile range. **Upper panel** shows group distances of physico-chemical characteristics **middle panel** distances of the community structure (ARISA), and **lower panel** the distances of bacteria function (Enzymes). Groups are split by catchment, season and water source as indicated on the x-axes, water source is indicated by brackets. Letters show significant differences based on Tukey's HSD ($P<0.05$). ND: no data.

Figure 3

NMDS of enzymatic activities. Dots indicate individual sites. The size of dots is relative to the sum of logarithms of all measured enzymes standardized to OM. Orange dots correspond to kryal sites and yellow dots to krenal sites. Dispersion-ellipses depict the standard error of weighted average scores of catchment groupings (Macun = M, Loetschental = L, Val Roseg = VR, confidence limits = 0.95). Environmental variables and bacterial OTU's are fitted as arrows. Projections of sites on fitted environmental vectors (arrows) show maximum correlation with the corresponding variable and vector lengths indicate strengths of gradient. Significantly fitted vectors are indicated by blue arrows for environmental variables ($P<0.05$ = dark blue, $P<0.05$ = light blue).

Figure 4

NMDS of ARISA profiles. Dots indicate individual sites. The size of dots is relative to the number of OTU's at a site. Bright blue dots correspond to kryal sites and dark blue dots to krenal sites. Dispersion-ellipses depict the standard error of weighted average scores of water source (kryal, krenal) within catchment groupings (Macun = M, Loetschental = L, Val Roseg = VR, confidence limits = 0.95). Environmental variables, enzyme activity and relative occurrence of bacterial groups and bacteria domains are fitted as arrows. Projections of sites on fitted environmental vectors (arrows) show maximum correlation with corresponding variable and vector lengths indicate strengths of gradient. Significantly fitted vectors ($P<0.05$) are indicated by dark blue arrows for environmental variables, orange for enzymatic activities.

Figure 1

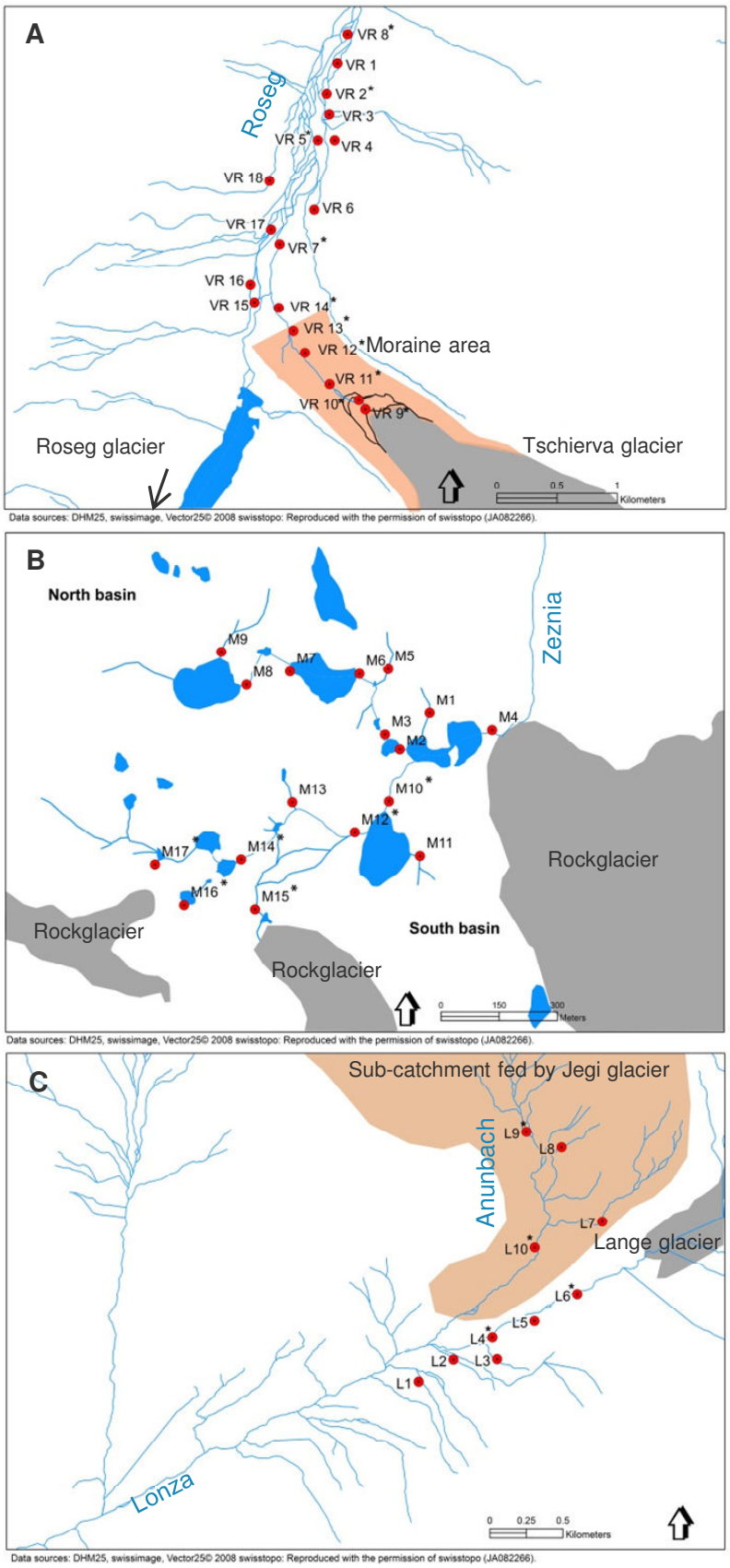

A
VR 8*
VR 1
VR 2*
VR 3
VR 5*
VR 4
Roseg
VR 18
VR 6
VR 17
VR 7*
VR 16
VR 15
VR 14*
VR 13*
VR 12*
Moraine area
VR 11*
VR 10*
VR 9*
Roseg glacier
Tschierva glacier
0 0.5 1
Kilometers
Data sources: DHM25, swissimage, Vector25© 2008 swisstopo: Reproduced with the permission of swisstopo (JA082266).
B
North basin
Zeznia
M9
M8
M7
M6
M5
M1
M3
M4
M2
M13
M10*
M12*
M17*
M14*
M11
M16*
M15*
Rockglacier
Rockglacier
South basin
Rockglacier
0 150 300
Meters
Data sources: DHM25, swissimage, Vector25© 2008 swisstopo: Reproduced with the permission of swisstopo (JA082266).
C
Sub-catchment fed by Jegi glacier
Anunbach
L9*
L8
L7
L10*
Lange glacier
L6*
L5
L4*
L2
L3
L1
Lonza
0 0.25 0.5
Kilometers
Data sources: DHM25, swissimage, Vector25© 2008 swisstopo: Reproduced with the permission of swisstopo (JA082266).

Figure 2

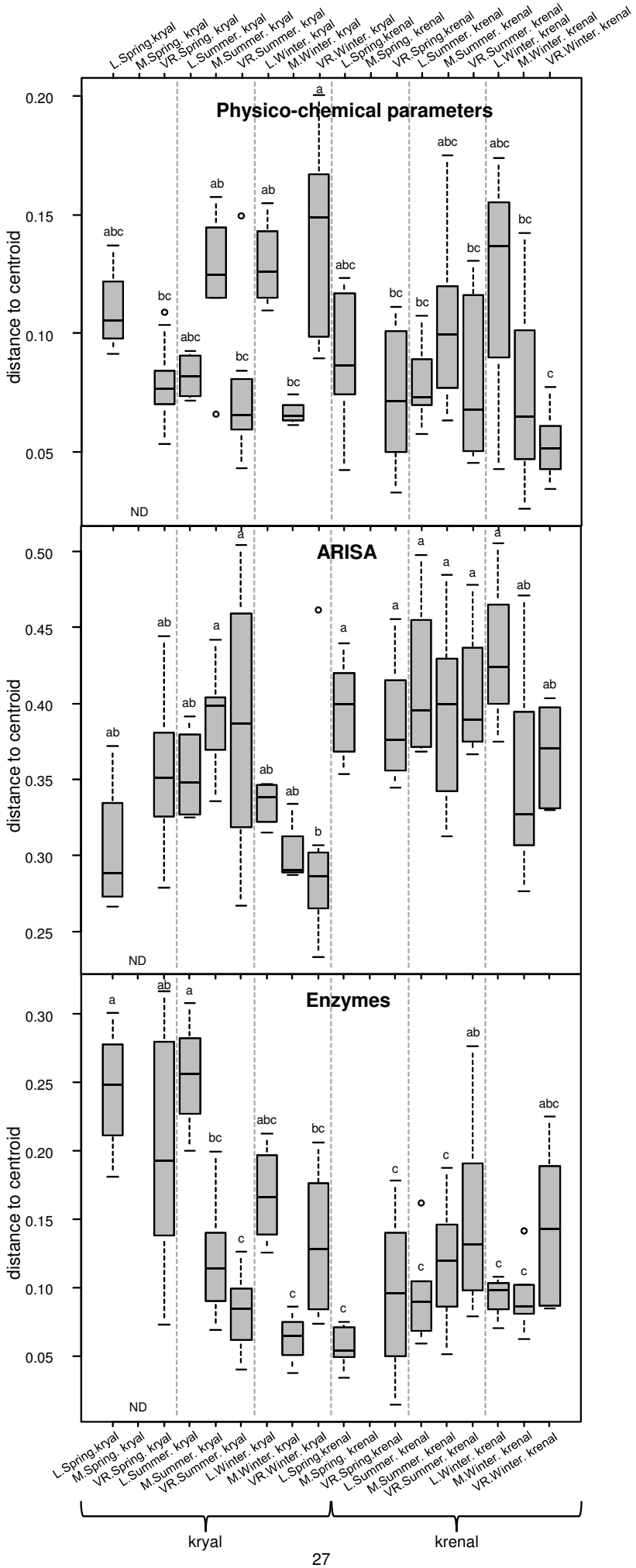
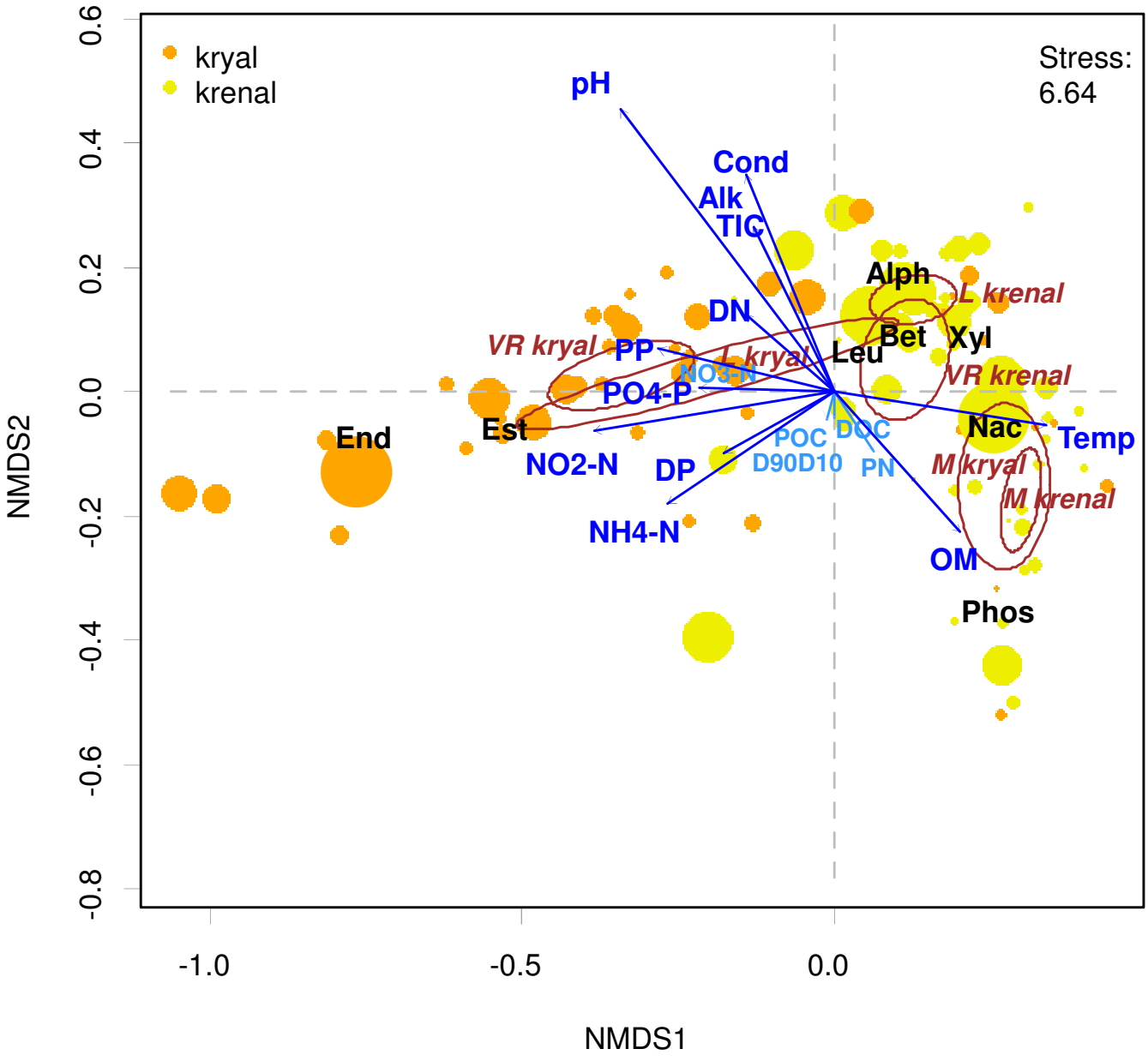

L.Spring.kryal
M.Spring. kryal
VR.Spring. kryal
L.Summer. kryal
M.Summer. kryal
VR.Summer. kryal
L.Winter. kryal
M.Winter. kryal
VR.Winter. kryal
L.Spring.krenal
M.Spring. krenal
VR.Spring.krenal
L.Summer. krenal
M.Summer. krenal
VR.Summer. krenal
L.Winter. krenal
M.Winter. krenal
VR.Winter. krenal
Physico-chemical parameters
ARISA
Enzymes
distance to centroid
ND
kryal
krenal

Figure 3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krenal
Stress:
6.64
pH
Cond
Alk
TIC
Alph
L krenal
DN
Bet
Xyl
VR kryal
PP
L kryal
Leu
VR krenal
NO3-N
PO4-P
End
Est
POC
DOC
Nac
Temp
NO2-N
DP
D90D10
PN
M kryal
M krenal
NH4-N
OM
Phos
NMDS1
NMDS2
-1.0
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0.0
0.6
0.4
0.2
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-0.4
-0.6
-0.8

Figure 4

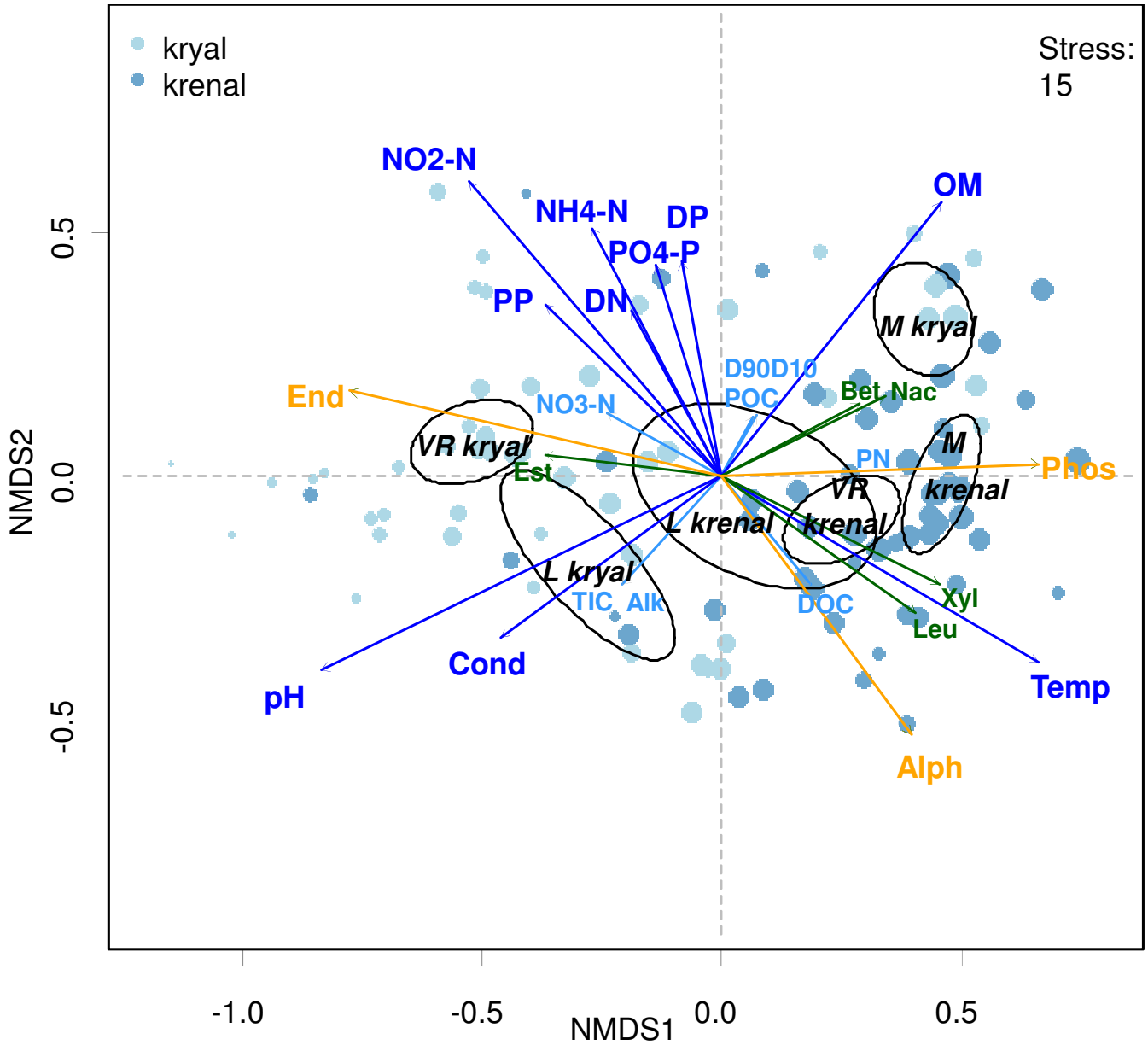

kryal
krenal
Stress:
15
NO2-N
NH4-N
DP
PO4-P
PP
DN
OM
M kryal
D90D10
POC
End
NO3-N
Bet Nac
VR kryal
Est
PN
M
krenal
Phos
VR
krenal
L krenal
L kryal
TIC
Alk
DOC
Xyl
Leu
Cond
pH
Temp
Alph
NMDS1
NMDS2
-1.0
-0.5
0.0
0.5
0.5
0.0
-0.5

Table 1 Characteristics of the three alpine catchments

Catchment	*Val Roeg*	*Loetschental*	*Macun*
Position	9°53'53''E, 46°29'24''N	07°49'03''E, 46°25'08''N	10°07'31''E, 46°43'51''N
Altitude [m.a.s.l]	1766-4049	1375-3200	2616-3046
Catchment Area [km^2], (% glaciated)	66.5 (30.1)	77.8 (36.5)	3.6
Annual precipitation [m]	1.6	1.1	0.9
Mean discharge [$m^3\ s^{-1}$]	28.5	37.2	ND
Mean water temperature of Main channel [°C] (range)	3.6 (1 - 12)	4 (0.1-10.9)	ND
Geology, dominating minerals	Crystalline bedrock, diorite, granite	Crystalline bedrock, amphibolite, gneiss	Crystalline rock, ortho-gneiss

Abbreviation: ND, no data

Table 2 Enzymes analyzed in this study, substrate used for assays and their biogeochemical functions

Enzyme (abbreviation)	*Substrate analogue*	*Acquiring element*	*Target*	*Function in ecosystem*
α-Glucosidase (Alph)	4-MUF-α-D-glucoside	Carbon	α-1,4- and 1,6-glucosidic linkages	Starch degradation
β-Glucosidase (Bet)	4-MUF-β-D-glucoside	Carbon	β-1,4-glucans	Cellulose degradation
β-Xylosidase (Xyl)	4-MUF-β-D-xylopyranoide	Carbon	Xylose residues	Hemicellulose degradation
Esterase (Est)	4-MUF-acetate	Carbon	Small ester containing molecules	Glyceride hydrolization
N-acetyl-glucosaminidase (Nac)	4-MUF-N-acetyl-β-D-glucosaminide	Nitrogen	1,4-β-linkages of glucosamines	Chitin degradation
Leucine aminopeptidase (Leu)	L-leucine-7-amido-4-methylcoumarin	Nitrogen	Hydrophobic amino acids from N terminus	Peptide degradation
Endopeptidase (Epep)	4-MUF-4-guanadinoenzoate	Nitrogen	Peptide bonds	Peptide degradation
Phosphatase (Phos)	4-MUF-phosphate	Phosphorous	Phosphomono- and diester	Protein, Nucleotide degradation

Table 3 Relative importance of multiple linear regression of enzymatic activities standardized to OM and physico-chemical parameters, underlined values are contributing negatively to the distinct enzyme activity, values are scaled to 100%, models were selected with AIC, see text for abbreviations

	Physico-chemical parameters														
Enzyme	Temp	Cond	pH	D90D10	DOC	POC	TIC	Alk	NH_4-N	NO_2-N	NO_3-N	PN	PO_4-P	DP	PP
Alph	0.029		0.619***							0.115*		0.049**	0.058***		0.13***
Bet			0.398***	0.048*						0.101		0.094***	0.087***	0.036	0.236***
Xyl	0.099*		0.309***	0.092*						0.148*		0.071**	0.111***		0.171***
Nac			0.096**	0.097								0.091**	0.066*	0.061	0.589***
Est	0.037*		0.373***	0.017*	0.006	0.02*	0.16	0.147*		0.04	0.025*		0.024*		0.149***
Leu		0.179	0.519***	0.056*						0.063*		0.058**	0.03**		0.096***
End			0.353*			0.024***		0.284***			0.033	0.014*		0.059**	0.233***
Phos	0.091	0.097**	0.051**	0.099*			0.076	0.124*	0.038	0.157*		0.055*			0.212*

* P<0.05

** P<0.01

*** P<0.001

Table 4 Procrustes analysis of community-, functional- and physico-chemical characteristics based NMDS. Given are r-values (correlations) of the symmetric procrustes rotation, EF = enzymatic functioning, Env = physico-chemical parameters, BCC = bacterial community composition

	Val Roseg				*Loetschental*				*Macun*			
	Total				Total				Total			
	EF		*Env*		*EF*		*Env*		*EF*		*Env*	
BCC	0.668***		0.597***		0.551***		0.426*		0.431*		0.532**	
	kryal			krenal	kryal			krenal	kryal			krenal
	EF	Env	EF	Env	EF	Env	EF	Env	EF	Env	EF	Env
BCC	0.558***	0.378*	0.332	0.494*	0.84***	0.681*	0.43	0.45	0.358	0.555	0.506*	0.597**

* P<0.05

** P<0.01

*** P<0.001

Supplementary information

Material and methods

Study floodplains

The Val Roseg catchment is situated in the eastern Swiss Alps and belongs to the lower austroalpine Bernina nappe (9°53'53''E, 46°29'24''N). Geology consists of crystalline bedrock composed mainly of diorite and granite (Malard *et al.,* 1999a). It covers an area of 66.5 km^2 from which 30.1% is glaciated. Annual precipitation rates are approximately 1.6 m, with around 50% as snow. The altitude of the catchment ranges from 1766 to 4049 m a.s.l., and the study area ranged from 2009 to 2336 m a.s.l.

The Roseg River is an 11.3-km long second-order tributary of the river Inn, which drains into Danube. Average annual discharge and water temperature are 28.5 $m^3 s^{-1}$ and 3.6°C (range0.1 to 12.6°C). Approximately 30% of the water volume of the Roseg River is fed by water from two valley glaciers, the Roseg glacier and Tschierva glacier, both of which have retreated continuously over the last century (Maisch 1988, Tockner *et al.,* 1997, Malard *et al.,* 2000a, Tockner *et al.,* 2002, 2010). Permanent flowing first-order tributaries contribute groundwater and snowmelt to the Roseg with peak flows during spring and summer (Malard *et al.,* 2000a). The channel network within the floodplain shows a distinct contraction in winter caused by the freezing of glacial water.

The Loetschental catchment is in the Rhone-Valley in the southwest part of the Swiss Alps (07°49'03"E, 46°25'08"N), harboring the second-order kryal stream Lonza which drains into the Rhone. It is part of the old crystalline Aare massif that is dominated by amphibolite and gneiss (Labhart 1998). The altitude of the 77.8 km^2 valley ranges from 1375 to 3200 m a.s.l. (Schmidt *et al.,* 2009, BAFU 2010), and the study area was located between 1929 to 2210 m a.s.l. Approximately 36.5% of the valley is glaciated by the Lang glacier and the Jegi glacier. The River Lonza shows an average discharge and water temperature of 37.2 $m^3 s^{-1}$ and 4.0°C (range of 0.1 to 10.9°C)(BAFU 2010). The kryal tributary fed by the Jegi glacier (Anunbach) and several first-order krenal tributaries drain into the Lonza within the study area. Most tributaries run dry during winter and the channel network experiences a similar periodical contraction as the Roseg cannel network.

The Macun Lakes region is a high alpine cirque situated in the Swiss National Park, and located in the mid-eastern part of the Swiss Alps (10°07'31"E, 46°43'51"N). It belongs to the upper austroalpine Silvretta nappe and geology consists mainly of crystalline rock dominated by orthogneiss. The catchment is divided into a southern and northern basin that differs in their water source (Robinson and Matthaei 2007). The northern basin is mainly groundwater and snowmelt fed, whereas the southern basin is fed mostly by rock glaciers. This catchment also experiences contraction of surface channels in winter (Robinson and Matthaei 2007).

Details on bacterial total cell numbers

A 0.5 ml aliquot of collected sediment (n=3) was suspended in 1.11 ml paraformaldehyde (2%, final concentration) in an Eppendorf tube and fixed for 24 h at 4°C followed by three washing steps with 1 x PBS and 5 min centrifugation at 10,000 g between washing steps. Samples were then stored at -20°C in a 1:1 mix of PBS/ethanol until further processing (Pernthaler *et al.,* 2001). Weight of the sediment, tube and the storage solution was measured. Attached bacteria were brought into suspension by sonication (Branson Digital Sonifier 250, Danbury, USA, 5-mm tapered microtip, actual output of 20 W) using 1-s sonication pulses for 30 s. After vortexing the sample for 7 s, a short spin centrifugation using a table top centrifuge for 5 s was performed to remove coarse particles interfering with downstream sample processing. The supernatant was transferred into a new Eppendorf tube and served as a template for total cell counting of 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich Co) stained cells. The remaining sediment was dried for 24 h at 60°C and weighed with and without the Eppendorf.

A 10 to 60 µl aliquot of template solution was pipetted into 5 ml sterile ultrapure water (MQ) and stained with DAPI (1 µg ml^{-1} final concentration) for 7 min followed by filtration onto a black polycarbonate filter (0.2-µm pore size, 25-mm diameter, Millipore, Molsheim, GTBP02500) by applying a gentle vacuum (Porter and Feig 1980). Filters were air-dried and embedded into citifluor AF1 (Linaris Biologische Produkte, Wertheim, Bettingen). An epifluorescence microscope (Leica Microsystem, DMI6000b) was used to take a minimum of 16 photographs of each stained filter to ensure an equal distribution and an appropriate number of bacterial cells (800+) for counting. Photographs were analyzed using the CellC software (Selinummi *et al.,* 2005) or counted

manually if background fluorescence interfered with the automated counting routine. Cell numbers were then standardized to the dry mass of the initially suspended sediment by using the weights determined during procession.

Details on enzyme assays

Approximately 10 g of sediment sample (n=3) were dissolved in 10 ml MQ and vortexed for 1 min to suspend the associated biofilms. A 150-µl aliquot was then transferred into a 96 well microplate and 100 µl of a 1 mM substrate stock solution was added to get a final substrate concentration of 400 µM (Findlay *et al.,* 2001). The remaining sediment and MQ were dried at 60°C for 48 h to measure the dry weight subsequently used to calculating the OM content. Fluorimetric enzyme assays were performed directly after adding the substrate for up to 24 h using a microplate reader (Tecan Infinite® 200, Switzerland). The excitation wavelength was set at 365 nm and fluorescence emission was measured at 445 nm. Plates were incubated on a plate shaker at 15°C between measurements. All fluorescence values were corrected for quenching by adding known quantities of free Methylumbelliferone (MUF) to the samples and to MQ and bicarbonate buffer blanks. Reaction rates were calculated using the linear part of the fluorescence reaction curve. Potential enzyme activities were standardized to nmol substrate g^{-1} OM h^{-1}.

Details on bacterial community fingerprinting

Bacterial community structure was assessed by ARISA. Frozen samples (n=2) were extracted using the PowerSoil DNA isolation Kit (MoBio, Carlsbad) following manufacturer's instructions. DNA was amplified using the fluorescein (6-FAM) labeled universal forward primer 1406f-6FAM (16S rRNA gene, 5'-FAM-TGYACACACCGCCCGT-3', Y=T,C) and the bacteria specific reverse primer 23Sr (5'-GGGTTBCCCCATTCRG-3', B=G,T,C, R=G,A)(Yannarell *et al.,* 2003). PCR was performed using a TProfessionalthermocycler (Biometra GmbH, Göttingen) in a final reaction volume of 25 µl with a mix of 1x GoTaq®Flexi buffer, 3 mM $MgCl_2$, 0.25 mM each of dNTP, 0.05 U μl^{-1} of GoTaq®Flexi DNA Polymerase (Promega, Switzerland), 0.25 mg ml^{-1} bovine serum albumin (Sigma-Aldrich, Switzerland), 0.4 µM of each primer (Microsynth, Switzerland), and 1 µl of template DNA. The reaction mix was initially denatured for 2 min at 94°C followed by primer annealing at 55°C for 35 s and extension of 2 min at 72°C. Denaturation time was then reduced to 35 s and the cycle was repeated 29 times followed by a final extension for 2 min at 72°C. ARISA fragment analysis was performed as described in Bürgmann *et al.* (2011). Briefly, a 1 µl aliquot of PCR product was mixed with 9 µl of HiDi formamide and 0.5 µl Liz1200 size standard (Applied Biosystems, Switzerland) and denatured prior to capillary electrophoresis on a PCR thermocycler for 3 min at 95° C and subsequently placed on ice. Denaturing capillary electrophoresis was performed on a 3130XL Capillary Genetic Analyzer (Applied Biosystems, Switzerland) equipped with a 50-cm capillary using POP-7 polymer. ARISA fragments were analyzed with the Southern size-calling method with a background cut-off level of 50 fluorescence units. Binning of peaks was done with automatic and interactive binning R scripts (Ramette 2009) leading to relative fluorescence intensity of peaks between 200 and 1400 bp and mean intensities of the extracted samples (n=2) was used for subsequent analysis.

Details on data analysis

Environmental factors, cell abundance was tested for homogeneity using a Bartlett-Test. Comparison of environmental variables, cell abundance and enzymatic activity between catchments, water source and season were done using three-factor ANOVA (Type I SS) followed by Tukey's HSD when differences were significant. Catchment, water source and season were treated as fixed factors in the ANOVA. Akaike information criterion (AIC) model reductions were performed for confirmation of non-significant model terms. Normality of residuals was assured by performing a Shapiro Willk's test and examining the QQ-plot of the residuals. If one of the assumptions was violated, data were transformed, after checking with the Box-Cox-Method (Krambeck 1995), by $\ln(x+1)$ or $x^{(Max\log -Likelihood)}$. Percentage values were $\arcsin(\sqrt{x})$ transformed prior to analysis. In case of non-conformity after transformation, a non-parametric Kruskal-Wallis test followed by multiple comparisons was performed instead of ANOVA.

Community fingerprinting results, enzymatic activities and environmental data were analyzed using NMDS in combination with vector and factor fitting. Untransformed enzyme activities and environmental parameters were used. Significance of fitted vectors and factors was tested by a permutation test (999 permutations) and visualized as biplots and dispersion ellipses in the NMDS ordinations. Generalized additive models were fitted on the NMDS for raw physico-chemical data to check linearity of fitted vectors (biplots) and used to assess the importance of single physico-chemical variables potentially constraining the BCC or EF pattern (Bennion *et al.,* 2011). Physico-chemical variables were therefore interpolated with the function ordisurf() from the vegan

package in R (Oksanen *et al.,* 2011). Shortly, the function fits a smooth surface using thinplate splines and the percentage of variance of each factor explained by the surface can be seen as a measure of how well an environmental variable explains the a priori and unconstraint NMDS pattern. The advantage of this approach is that linear projections of environmental variables (biplots) in the ordination space may not be de facto ideally linear as i.e. in constraint ordinations, thus takes non-linear relationships better into account.

The totally explained variance of the physico-chemical factors on BCC and EF was assessed by a global RDA model incorporating all samples and physico-chemical parameters. Canonical coefficients (i.e. the regression coefficients) of the explanatory variables of the first two axes were calculated. This allows assessing which variables are most important to explain EF or BCC structure on these two axes and can be qualitatively compared to the explained deviance of the respective GAM model. RDA based variation partitioning were performed to evaluate the influence of assessed chemical and physical (temperature and D90D10) factors on bacteria structure and function as a whole and within the specific water systems. For both approaches, variation inflation factors were calculated and physico-chemical factors which had a value above 20 were removed before a forward selection was performed. This minimizes collinearity of respective variables with other variables included in the analysis. Unique fractions of RDA were tested by an ANOVA like permutation test. Reported are the adjusted R^2 (Peres-Neto *et al.,* 2006, Blanchet *et al.,* 2008a). RDA based analysis were performed with Hellinger transformed ARISA and enzymatic activity data.

Permutational multivariate analysis of variance (PERMANOVA) was used to assess the influence of water source, catchment and season (full factorial model) on community and enzyme activity structure (Anderson 2001).

Correlation of single enzymes to physico-chemical factors were tested by multiple linear regression. The models were selected by AIC, significance of single predictors was tested by permutational ANOVA (Type III SS) and relative importance of predictors in the linear model was assessed using the lmg metric (Chevan and Sutherland 1991, Grömping 2006). The enzymatic activities standardized to OM were used for the models without OM as a predictor incorporated. This prevents autocorrelation. Physico-chemical factors were $\ln(x+1)$ transformed.

A pairwise PERMANOVA comparison the catchments, seasons and water sources was also performed for EF and BCC structures to assess the coupling of structural and functional measures of bacterial communities. This was done by giving P-values higher and lower than 0.05 a value of 0 and 1, respectively, and then summing the corresponding pairs of structure and function. Sums of zero and 2 indicate a coupling of structural and functional measures, whereas a value of 1 indicates a decoupled relationship. This assessment was supported by a Mantel test and a procrustes analysis. The Mantel test between ARISA and enzyme activities was based on 999 permutations (Mantel 1967). The procrustes analysis of the corresponding NMDS ordinations leads to m^2 statistic, which is used as a measure of congruence of the two ordinations, and the procrustes correlation $r = \sqrt{1 - m^2}$. Configurations of NMDS were scaled to equal dispersion. Non-randomness between the two configurations was tested with the function protest by 999 permutations (Gower 1975, Digby and Kempton 1987, Jackson 1995, Peres-Neto and Jackson 2001). Procrustes analysis was additionally performed for ARISA and enzymatic activities vs. environmental variables NMDS ordinations.

Multivariate homogeneity of groups dispersion (MHGD), as an analogue of Levene's test for homogeneity of variance, was performed to assess beta diversity of enzyme activities, fingerprinting profiles and physico-chemical characteristics. Generally, multivariate dispersion (variance) can be calculated by the distances of group members to a group centroid in multivariate space. Differences in dispersions can then be tested by ANOVAs. Bray-Curtis dissimilarities of transformed and standardized datasets were reduced from their original distances to principal coordinates (PcoA) which embeds them within an Euclidian space. The average distances to centroids were then calculated within groups, thus are based on principal coordinate axes rather than the original distances. Differences between groups were assessed using a permutational ANOVA test (999 permutations) followed by a Tukey's HSD (Anderson *et al.,* 2006). NMDS, MHGD and PERMANOVA were all based on Bray-Curtis dissimilarity matrices calculated from the Wisconsin double standardized relative fluorescence intensity of ARISA profiles, Wisconsin standardized square-rooted enzyme activities and $\ln(x+1)$ transformed environmental variables (Bray and Curtis 1957). Shannon diversity index was calculated for OTU's (operational taxonomic units).

All analyses were done using the vegan, relaimpo, stats and mgcv package in R (R Development Core Team, 2011, Oksanen *et al.,* 2011).

Detailed results of physico-chemical parameters

There was a strong influence of catchment, water source and season on water temperature (ANOVA: $F_{2,89}$=18.53, $F_{1,89}$=32.98 and $F_{2,89}$=8.40, respectively, P<0.001). Water temperatures in the three catchments showed similar patterns. Kryal (glacial) systems were colder (range: 0.3 to 11.9°C, mean: 4.3°C) during the study period than groundwater (krenal) systems (range: 2.3 to 20.7°C, mean: 8.5°C, Tukey's HSD: P<0.001). Val Roseg streams were generally colder than streams in the other two catchments (Tukey's HSD: P<0.001). Temperature was lower in winter compared to summer and spring (Tukey's HSD: P<0.05). The krenal system in Val Roseg had the lowest mean temperatures compared to Macun and Loetschental krenal sites, which did not differ (5.8 vs. 10.1 and 10.5°C, respectively, ANOVA: $F_{2,52}$=5.91, P<0.001, Tukey's HSD: P<0.05).

Conductivity showed significant interactions between catchment and water source, catchment and season and water source and season (ANOVA: $F_{2,89}$=5.70 and $F_{3,89}$=2.90 and $F_{2,89}$=5.80 respectively, P<0.05). There were significant differences in conductivity between kryal and krenal systems in the Loetschental (mean krenal: 89.9±48.5, mean glacial water: 44.74±23.3 µS cm^{-1}, Tukey's HSD: P<0.01). Lowest conductivity values were found in the Macun catchment (mean: 7.9 ± 3.4µS cm^{-1}, Tukey's HSD: P<0.01) and highest values in Loetschental and Val Roseg, which did not differ (69.8 ± 44.9 and 62.6 ± 34.5 µS cm^{-1}, respectively, ANOVA: $F_{2,89}$=200.76, P<0.001, Tukey's HSD: P=0.64).

The three catchments were clearly distinct in respect to sediment pH with lowest mean pH measured in Macun followed by Loetschental and Val Roseg (mean: pH 4.86, 6.64 and 7.27, respectively, ANOVA: $F_{2,89}$=558.08, P<0.001, Tukey's HSD: P<0.001). Sediment pH showed a significant interaction between catchments and water source with a significant difference between water systems (kryal vs. krenal) only in Val Roseg (ANOVA: $F_{2,89}$=6.82, P<0.01, Tukey's HSD: P<0.001).

Macun had higher amounts of OM (mean: 2.54 ± 2.43% OM dw^{-1}) in the sediment compared to the other two catchments, which were similar (Loetschental: 0.53 ± 0.36% OM dw^{-1}, Val Roseg: 0.55 ± 0.64% OM dw^{-1}, Kruskal-Wallis: H=46.7, df=2, P<0.001, multiple comparison: P<0.01). OM was generally lower in the kryal than in the krenal system (mean: 0.76 and 1.32% OM dw^{-1}, respectively, Kruskal-Wallis: H=19.07, df=1, P<0.01, multiple comparison: P<0.01).

DOC was lower in Val Roseg compared to Macun but not to Loetschental, whereas Macun and Loetschental did not differ from each other (mean: 0.95 ± 1.55 mg DOC ml^{-1}, 2.16 ± 3.51 and 2.37 ± 3.93 mg DOC ml^{-1}, respectively, Kruskal-Wallis: H=10.59, df=2, P<0.01, multiple comparison: P<0.01). There was no significant difference in DOC between the two water types in each catchment (Kruskal-Wallis: H=3.33, df=1, P=0.07). Winter DOC concentrations were higher compared to those in summer (Kruskal-Wallis: H=24.69, df=2, P<0.001, multiple comparison: P<0.01). Loetschental kryal DOC concentrations were higher in spring than the ones in summer (Kruskal-Wallis: H=61.43, df=15, P<0.001, multiple comparison: P<0.01)

POC concentrations in Val Roseg differed from Macun but not from Loetschental, (Kruskal-Wallis: H=12.22, dF=2, P<0.01, multiple comparison: P<0.01). More detailed, there was a significant difference in POC in krenal sites between Macun and Val Roseg (mean: 1.22 ±1.34 and 0.21 ± 0.12 mg C l^{-1}, respectively, Kruskal-Wallis: H=21.96, df=5, P<0.001, multiple comparison: P<0.01).

There was a significant interaction between catchment and season in TIC concentrations (ANOVA: $F_{3,89}$=10.26, P<0.001). The Val Roseg catchment showed seasonal differences with lower TIC concentrations in summer than in winter and spring (Tukey's HSD: P<0.01). TIC differed between catchments and was lowest in Macun (mean: 1.77 ± 1.2 mg C l^{-1}) and highest in Roseg and Loetschental (mean: 5.39 ± 2.33 mg C l^{-1} and 4.02 ± 1.43 mg C l^{-1}, respectively, ANOVA: $F_{2,89}$=67.90, P<0.001, Tukey's HSD: P<0.05). Krenal systems had higher TIC values than kryal systems (ANOVA: $F_{1,89}$=8.12, P<0.01).

Alkalinity showed the same pattern as TIC: Macun had lower alkalinity than the other two catchments (Kruskal-Wallis: H=42.07, df=2, P<0.001, multiple comparison: P<0.01). Within the Val Roseg catchments alkalinity was lowest in summer (Kruskal-Wallis: H=15.63, df=2, P<0.001, multiple comparison: p<0.01).

NH_4-N showed no differences between catchments (Kruskal-Wallis: H=0.48, df=2, P=0.79), but lower values for krenal than kryal channels in Val Roseg (Kruskal-Wallis: H=22.63, df=1, P<0.001).

NO_2-N differed between the two water sources in Val Roseg (krenal mean: 0.53 ± 0.12 µg N l^{-1}, kryal mean: 2 ± 1.04 µg N l^{-1}, Kruskal-Wallis: H=52.66, df=5, P<0.001, multiple comparison: P<0.01). Macun showed a seasonal decrease in NO_2–N towards winter (Kruskal-Wallis: H=18.06, df=1, P<0.001).

Val Roseg had higher NO_3-N concentrations than the other two catchments (Kruskal-Wallis: H=19.42, df=2, P<0.001, multiple comparison p<0.01). NO_3-N was lowest in summer (Kruskal-Wallis: H=26.19, df=2, P<0.001, multiple comparison p<0.01). Krenal sites in Macun had lower NO_3-Nconcentrations compared to Macun and Val Roseg kryal sites (Kruskal-Wallis: H=32.78, df=5, P<0.001, multiple comparison p<0.05).

Val Roseg had the highest values of DON (Kruskal-Wallis: H=28.72, df=2, P<0.001, multiple comparison: P<0.01). Macun had the higher DON concentrations in the kryal system (Kruskal-Wallis: H=5.27, df=1, P<0.05) and had generally higher concentrations in winter (Kruskal-Wallis: H=4.15, df=1, p<0.05). Loetschental had lowest DON values in summer followed by winter and spring (Kruskal-Wallis: H=16, df=2, p<0.001, multiple comparison: p<0.05).

Macun had higher PN concentrations compared to the other catchments (Kruskal-Wallis: H=19.01, df=2, P<0.001, multiple comparison: P<0.01) and also differences between the two water systems (Kruskal-Wallis: H=9.1, df=1, P<0.01) with highest values in krenal streams. Water systems in Val Roseg also differed in PN concentrations (Kruskal-Wallis: H=10.54, df=1, P<0.01), except that PN was higher in kryal channels.

No difference was detected in DP between catchments (Kruskal-Wallis: H=5.22, dF=2, P=0.07). Glacial waters showed highest concentrations of DP (Kruskal-Wallis: H=8.63 ,dF=1, P<0.01), except for Macun where DP was the same for both systems (Kruskal-Wallis: H=0.13, df=1, P=0.72). Loetschental and Macun had lower DP in winter compared to spring and summer (Kruskal-Wallis: H=9.35 and 4.57, df=2 and 1, P<0.01 and 0.05, respectively).

PP was lower in Macun kryal waters than in the other two catchments (Kruskal-Wallis: H=14.29, df=2, P<0.001, multiple comparison: P<0.01). Kryal systems had higher PP concentrations compared to krenal systems in the Roseg catchment (Kruskal-Wallis: H=22.05, df=1, P=<0.001). Krenal sites had higher PP in Loetschental than in Roseg (Kruskal-Wallis: H=12.93, df=2, p<0.01, multiple comparison: P<0.01). A seasonal peak in PP during summer was visible in Val Roseg (Kruskal-Wallis: H=8, df=2, p<0.05, multiple comparison: P <0.05).

No difference in PO_4-P concentrations was detected between catchments (Kruskal-Wallis: H=2.63, df=2, P=0.27), although glacial waters generally had highest PO_4–P levels (Kruskal-Wallis: H= 10.04, df=1, P<0.01). Loetschental had higher PO_4–P concentrations in spring than winter (Kruskal-Wallis: H=8.56, df=2, P<0.05, multiple comparison: P<0.03).

The D90/D10 sorting coefficient did not show a significant difference between the catchments, water source or the season respectively (ANOVA: P>0.05).

The MHGD analysis showed an interaction of water source, season and catchment on beta diversity of physico-chemical characteristics (Permutation test: $F_{15,89}$=4.59, P<0.001). In particular, Roseg kryal waters showed higher beta diversity in winter compared to most krenal sites, except for Loetschental spring and winter and Macun summer (Tukey's HSD: P<0.05). Roseg kryal beta diversity in winter was also higher than the Val Roseg kryal systems in spring and summer and the Macun winter kryal system (Tukey's HSD: P<0.05).

Ordination of environmental parameters revealed differences in physico-chemical characteristics between water sources, catchments and season (PERMANOVA: $F_{1,89}$=21.22, $F_{2,89}$=24.59 and $F_{2,89}$=8.89, respectively, P<0.001). An interaction between catchment and season, and catchment and water source was apparent (PERMANOVA: $F_{3,89}$=5.04, and $F_{2,89}$=8.52, P<0.001). Physico-chemical characteristics were not different between Loetschental and Val Roseg krenal waters in summer and winter (PERMANOVA: $F_{1,13}$=2.31, P=0.06 and $F_{1,13}$=3.37, P>0.59, respectively). Macun showed no significant difference between water systems in summer or winter (PERMANOVA: $F_{1,17}$=1.33, P=0.24 and $F_{1,8}$=3.18, P=0.054, respectively). Loetschental krenal systems did not significantly differ from kryal waters in winter and spring (PERMANOVA: $F_{1,6}$=0.23, P=0.872, $F_{1,9}$=1.26, P=0.297, respectively). Loetschental krenal systems did not differ between spring and summer (PERMANOVA: $F_{1,11}$=1.67, P=0.147). Val Roseg showed no shift in physico-chemical characteristics between winter and spring in krenal systems (PERMANOVA:$F_{1,13}$=.16, P=0.375).

Detailed results of enzymatic activities

Alph had highest activities in krenal systems (ANOVA: $F_{1,89}$=28.87, P<0.001, Tukey's HSD: P<0.001) and lowest activities in Macun (ANOVA: $F_{2,89}$=17.70 P<0.001, Tukey's HSD: P<0.001).

Bet had a significant interaction between catchment, water and season (ANOVA: $F_{3,89}$=3.64, P<0.05), with krenal sites being more active than kryal sites in spring and summer in Val Roseg (Tukey's HSD: P<0.05). Roseg had higher Bet activities than the other two catchments (Tukey's HSD: P<0.05).

Xyl had a significant interaction between water, catchment and season, being higher in Roseg krenal sites than in Macun krenal sites in summer (ANOVA: $F_{3,89}$=2.94, P<0.05, Tukey's HSD: P<0.05). In spring, kryal sites in Val Roseg were lower in activities than krenal sites (Tukey's HSD: P<0.001). There was generally less Xyl activity in krenal sites in Macun than in the other catchments (ANOVA: $F_{2,89}$=4.98, P<0.01, Tukey's HSD: P<0.05), and krenal sites were more active than kryal sites in Loetschental (Tukey's HSD: P<0.05). Roseg and Loetschental had more Xyl activity than Macun (ANOVA: $F_{2,89}$=6.01, P<0.001, Tukey's HSD: P<0.05), and krenal sites had more activity then kryal sites (ANOVA: $F_{1,89}$=43.64, P<0.001, Tukey's HSD: P<0.001).

Est had different activities between the three catchments, being highest in Roseg, intermediate in Loetschental and lowest in Macun (ANOVA: $F_{2,89}$=85.93, P<0.001, Tukey's HSD: P<0.005).

Nac was lower in Loetschental compared to Val Roseg in summer (ANOVA: $F_{3,89}$=4.61, P<0.05, Tukey's HSD: P<0.05). In Val Roseg, krenal sites had higher activity than kryal sites, and also higher activity than krenal and kryal sites in Macun and kryal sites in Loetschental (ANOVA: $F_{2,89}$=5.66, P<0.01, Tukey's HSD: P<0.001, P<0.01, P<0.05 and P<0.001, respectively). The krenal systems showed generally higher Nac activity (ANOVA: $F_{1,89}$=33.14, P<0.001, Tukey's HSD: P<0.001).

Leu had different activities between water systems with higher values occurring in krenal sites (ANOVA: $F_{1,89}$=19.87, P<0.01) and highest activities in Roseg followed by Loetschental and Macun (ANOVA: $F_{2,89}$=25.24, P<0.001, Tukey's HSD: P<0.05).

End was highest in Val Roseg, intermittent in Loetschental and lowest in Macun (ANOVA: $F_{2,89}$=65.86, P<0.001, Tukey's HSD: P<0.001). Kryal systems had the highest End activities (ANOVA: $F_{1,89}$=14.85, P<0.001, Tukey's HSD: P<0.001).

In Val Roseg krenal sites Phos activity was higher compared to all other water systems within the three catchments except for Macun krenal system which did not differ (ANOVA: $F_{2,89}$=6.02, P<0.01, Tukey's HSD: P<0.05). Val Roseg and Macun were generally more active for Phos compared to Loetschental (ANOVA: $F_{2,89}$=4.73, P<0.05, Tukey's HSD: P<0.05), and krenal sites showed higher Phos activity then kryal sites (ANOVA: $F_{1,89}$=48.07, P<0.001, Tukey's HSD: P<0.001).

MHGD revealed an interaction between water source, season and catchment in beta diversity of enzymatic activities (Permutation test: $F_{15,89}$=7.13, P<0.001). Loetschental kryal sites had much higher beta diversity in spring and summer compared to the other sites at any time, except for Loetschental kryal sites in winter and Val Roseg krenal sites in summer and winter and Val Roseg kryal site in spring (Tukey's HSD: P<0.05). The Val Roseg kryal sites in spring had higher enzymatic beta diversity compared to Macun kryal channels during winter, Val Roseg kryal channel during summer and all krenal channels except for Val Roseg krenal sites in summer and winter (Tukey's HSD: P<0.05).

Ordination of enzyme activities showed that the Macun catchment differed in both water systems from krenal systems of the other catchments in all seasons (PERMANOVA: Macun vs. Roseg: $F_{1,48}$=13.64, P<0.001, Macun vs. Loetschental: $F_{1,41}$=25.88, P<0.001). Macun water systems were not significantly different in activity patterns (PERMANOVA: $F_{2,26}$=1,27, P=0.26), whereas the other two catchments showed strong separation in activity structures between krenal and kryal sites (PERMANOVA: Roseg: $F_{1,50}$=25.85, P<0.001, Loetschental: $F_{1,26}$=7.42, P<0.01). No differences could be detected between Loetschental and Roseg activities within each water system within any season (appendix Table 1). A seasonal shift in enzymatic pattern was present only in Roseg for both water systems (PERMANOVA: $F_{2,28}$=2.645, P<0.05 and $F_{2,21}$=2.137, P<0.05, respectively, appendix Table 1).

RDA analysis accounted 50.4% of variation in EF to physico-chemical factors (physical and chemical, R^2adj=0.504, $F_{5,99}$=22.15, P<0.001). See appendix Table 6 for canonical coefficients of the first two constraint axes. Variation partitioning revealed that the total contribution of chemical/physical factors on enzymatic activity was 37.1% / 0% of explained variation. The shared

fraction explained 13.3% of the variation in EF (Total R^2_{adj}=0.505, $F_{6,98}$=18.68, P<0.01, chemical fraction: R^2_{adj}=0.371, $F_{5,98}$=16.42, P<0.01, physical fraction R^2_{adj} =0.001, $F_{1,98}$=1.14, P=0.33, joint fraction R^2_{adj}=0.133, not testable). When variation partitioning was assessed for the two water sources independently, there was 40.6% of variation in EF explained by chemical factors, 0% by physical factors and 8.2 % by the shared fraction in the kryal systems (Total R^2_{adj}=0.479, $F_{5,44}$=10.01, P<0.01, chemical fraction: R^2_{adj}=0.406, $F_{4,44}$=10.35, P<0.01, physical fraction R^2_{adj} =-0.009, $F_{1,44}$=0.23, P=0.87, joint fraction R^2_{adj}=0.082, not testable). The variation partitioning within the krenal system revealed that none of the physical factors were kept in the model after forward selection. Thus, forward selected chemical factors were tested independently and explained 42.3% of the variation of enzymatic activities (Chemical factors: R^2_{adj}=0.424, $F_{3,51}$=14.23, P<0.001).

Detailed results of bacterial community structure and linked functions

The number of all 191 detected OTUs ranged from 28 at site VR12 in spring to 127 at site M13 in summer. There was a significant interaction between catchment and water source on OTU richness (ANOVA: $F_{2,89}$=4.19, P<0.05). The number of OTUs differed between water source in Val Roseg with a higher number of OTUs in krenal than kryal sites (Tukey's HSD: P<0.001). Loetschental had a lower number of OTUs in krenal sites than Val Roseg (Tukey's HSD: P<0.05) and a lower number of OTUs in kryal sites compared to krenal sites in Macun and Val Roseg (Tukey's HSD: P<0.05).

Beta diversity of MHGD showed a significant interaction between water source, season and catchment (Permutation test: $F_{15,89}$=3.53, P<0.001). Tukey's HSD revealed lower beta diversity in Val Roseg kryal sites in winter compared to all krenal systems except to Macun and Val Roseg krenal systems in winter. It was also different to Macun and Val Roseg summer kryal systems (Tukey's HSD: P<0.05).

RDA revealed that 19.5% of total variation in BCC was explained by forward selected environmental factors (physico-chemical, R^2_{adj}=0.195, $F_{9,95}$=3.80, P<0.001). See appendix Figure 7 for canonical coefficients of the first two constraint axes. Variation partitioning showed that the total contributions of chemical/physical factors on BCC were 12.2% / 1.2% of total variation. The shared fraction explained 5.5% of the variation (Total R^2_{adj}=0.190, $F_{8,96}$=4.04, P<0.01, chemical fraction: R^2_{adj}=0.122, $F_{7,96}$=3.22, P<0.01, physical fraction R^2_{adj} =0.012, $F_{1,96}$=2.50, P<0.01, joint fraction R^2_{adj}=0.055, not testable). Variation partitioning of physical and chemical parameters applied to the kryal systems showed 20.7% of the variation in community structure was accounted for solely by water chemistry (Total R^2_{adj}=0.263, $F_{7,42}$=3.50, P<0.01, chemical fraction: R^2_{adj}=.0.207, $F_{6,42}$=3.25, P<0.01, physical fraction R^2_{adj} =0.014, $F_{1,42}$=1.84, P<0.05, joint fraction R^2_{adj}=0.042, not testable). Krenal systems, in contrast, had just 9.3% of the variation in BCC explained by water chemistry (Total R^2_{adj}=0.112, $F_{6,48}$=2.13, P<0.01, chemical fraction: R^2_{adj}=0.093, $F_{5,48}$=2.21, P<0.01, physical fraction R^2_{adj} =0.014, $F_{1,48}$=1.79, P<0.01, joint fraction R^2_{adj}=0.004, not testable).

Enzyme activities of Phos and Alph were significantly situated towards krenal bacterial communities (fitting significance: P<0.01 and P<0.05 respectively, based on 999 permutations). Gradient directions tended towards Roseg and Loetschental for Alph and towards Macun for Phos. Xyl and Leu also were situated more towards krenal systems, but in a less pronounced manner (fitting significance: P<0.1, based on 999 permutations). Bet and Nac were not fitted significantly in the ordination (P=0.391 and P=0.252, respectively), although they have a weak gradient towards the Macun kryal system. Est and End enzyme activity fitted different from the other tested enzymes with a gradient direction towards kryal systems.

Ordination of enzyme activity structure underlines this functional separation between water sources, except for Macun (Figure 4). The tendency of generally higher Phos and Nac activity was apparent for the Macun catchment. Bet activity centroid, which showed a gradient towards Macun kryal systems in the community structure NMDS, was situated more near the Roseg and Loetschental krenal systems. This result is probably due to the weak environmental fitting power in the community structure ordination.

Procrustes analysis for community structure and environmental variables were correlated (r=0.525, P<0.01) when performed with all catchments. Correlation of single catchments of the same ordination showed maximum correlations for Val Roseg (r=0.597, P<0.001), Loetschental (r=0.426, P<0.05) and Macun (r=0.532, P<0.01). Correlations between enzymatic structure and environmental structure were r=0.492 (P<0.001) for all catchments, r=0.582 (P<0.001) for Loetschental, r=0.514 (P<0.001) for Val Roseg and r=0.334 (P=0.133) for Macun. See table 4 for procrustes test separated by water source within catchments and also between enzymatic and bacterial structure.

Refinement of individual dependency of enzymatic activity on environmental variables by multiple linear regression and relative importance metrics revealed that PP and NO_2-N had a negative influence (or correlation) on most of enzymes, whereas pH, D90D10, PN, PO_4-P, alkalinity and temperature positively influenced enzyme activities (Table 5).

Fitting single OTU's on the community function NMDS outcropped 108 OTU's out of 191 OTU's that could have been fitted with a permutation power of $P<0.05$, 74 OTU's with a power <0.01, 50 with power <0.001, 30 OTU's with power <0.0001, and 17 OTU's with a power of <0.00001 (100000 permutations). OTU's with fitting power <0.01 were split into a fraction showing a direction of gradient towards the kryal systems of Val Roseg and Loetschental and a fraction towards the Macun catchment. Only 4 OTU's showed a gradient towards Roseg and Loetschental krenal system with power $P<0.001$. One OTU could be fitted with a power below $P<0.0001$ towards this system (appendix Figure 2).

Supplementary information figure legends

Appendix Figure 1

NMDS of physico-chemical parameters. Dots indicate individual sites. Coding is equal to sites in figure 1 in the main manuscript, suffix letter indicate season (summer: A, winter: O, spring: J). Dark grey dots correspond to kryal sites and light grey dots to krenal sites. Dispersion-ellipses depict the standard error of weighted average scores of water source within catchment grouping (confidence limit=0.95).

Appendix Figure 2

NMDS of enzymatic activities. **Upper panel**: Dots indicate individual sites. The size of dots is relative to the sum of logarithms of all measured enzymes standardized to OM. Orange dots correspond to kryal sites and yellow dots to krenal sites. Dispersion-ellipses depict the standard error of weighted average scores of catchment groupings (confidence limits = 0.95). Environmental variables and bacterial OTU's are fitted as arrows. Projections of sites on fitted environmental vectors (arrows) show maximum correlation with the corresponding variable and vector lengths indicate strengths of gradient. Significantly fitted vectors are indicated by blue arrows for environmental variables ($P<0.05$ = dark blue, $P<0.05$ = light blue) and grey arrows for bacterial OTU's ($P<0.001$). **Lower panel:** Dots depicts individual sites. Coding is equal to sites in figure 1 in the main manuscript, suffix letter indicate season (summer: A, winter: O, spring: J).

Appendix Figure 3

NMDS of ARISA profiles. **Upper panel**: Dots indicate individual sites. The size of dots is relative to the number of OTU's at a site. Bright blue dots correspond to kryal sites and dark blue dots to krenal sites. Dispersion-ellipses depict the standard error of weighted average scores of water source (kryal, krenal) within catchment groupings (Macun = M, Loetschental = L, Val Roseg = VR) (confidence limits = 0.95). Environmental variables and enzyme activity are fitted as arrows. Projections of sites on fitted environmental vectors (arrows) show maximum correlation with corresponding variable and vector lengths indicate strengths of gradient. Significantly fitted vectors ($P<0.05$) are indicated by blue arrows for environmental variables, orange for enzymatic activities and red for bacterial domain and groups. **Lower panel**: Dots depict individual sites. Coding is equal to sites in figure 1 in the main manuscript, suffix letter indicate season (summer: A, winter: O, spring: J).

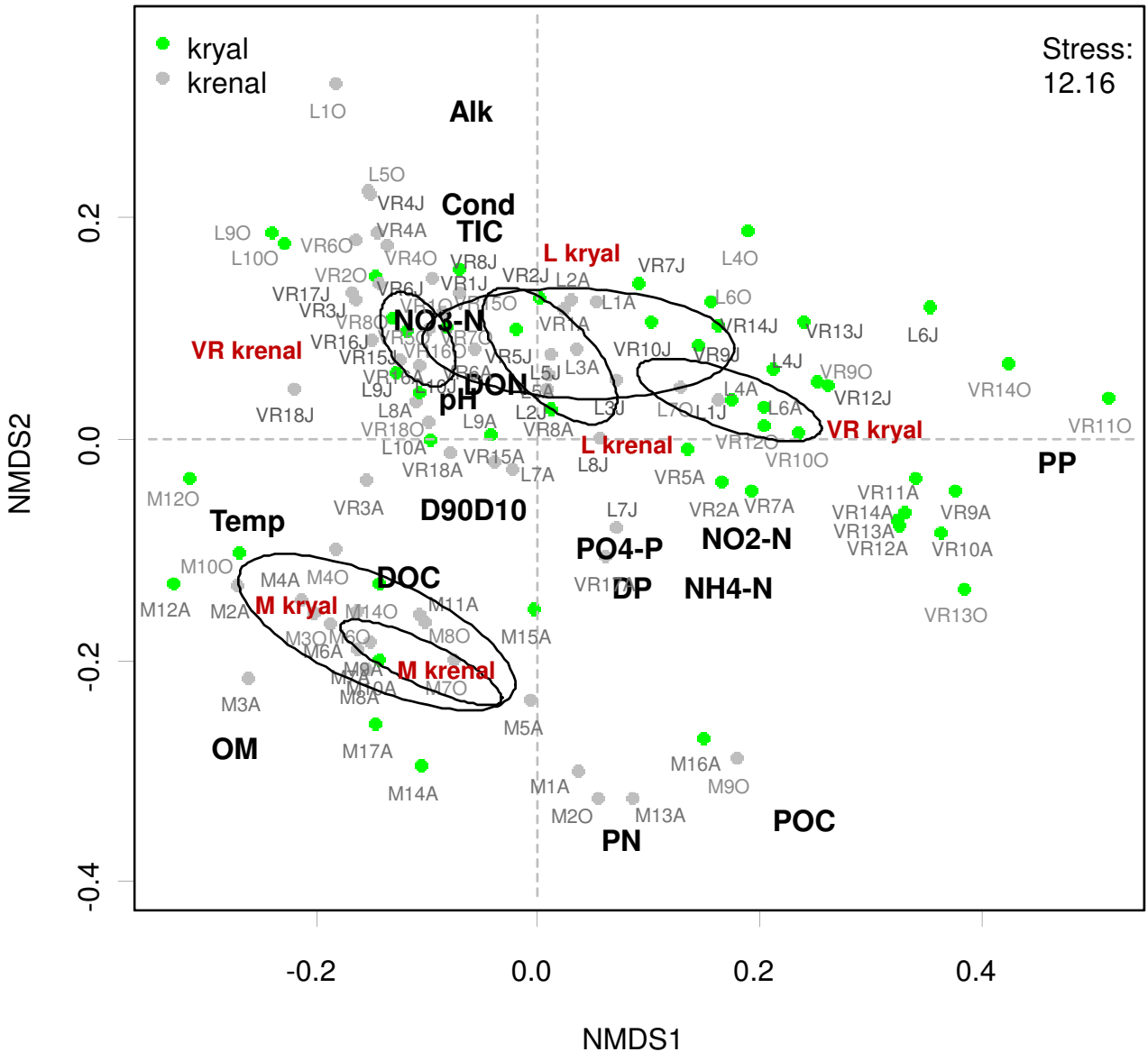
Appendix Figure 4a to 4c

NMDS of enzymatic activities with response surface as assessed by generalized additive models (GAM) for the measured physico-chemical variables. Each contour line is annotated with the specific value of the variable. The percentages of variances explained by variables are given and correspond to the values from appendix table 6.

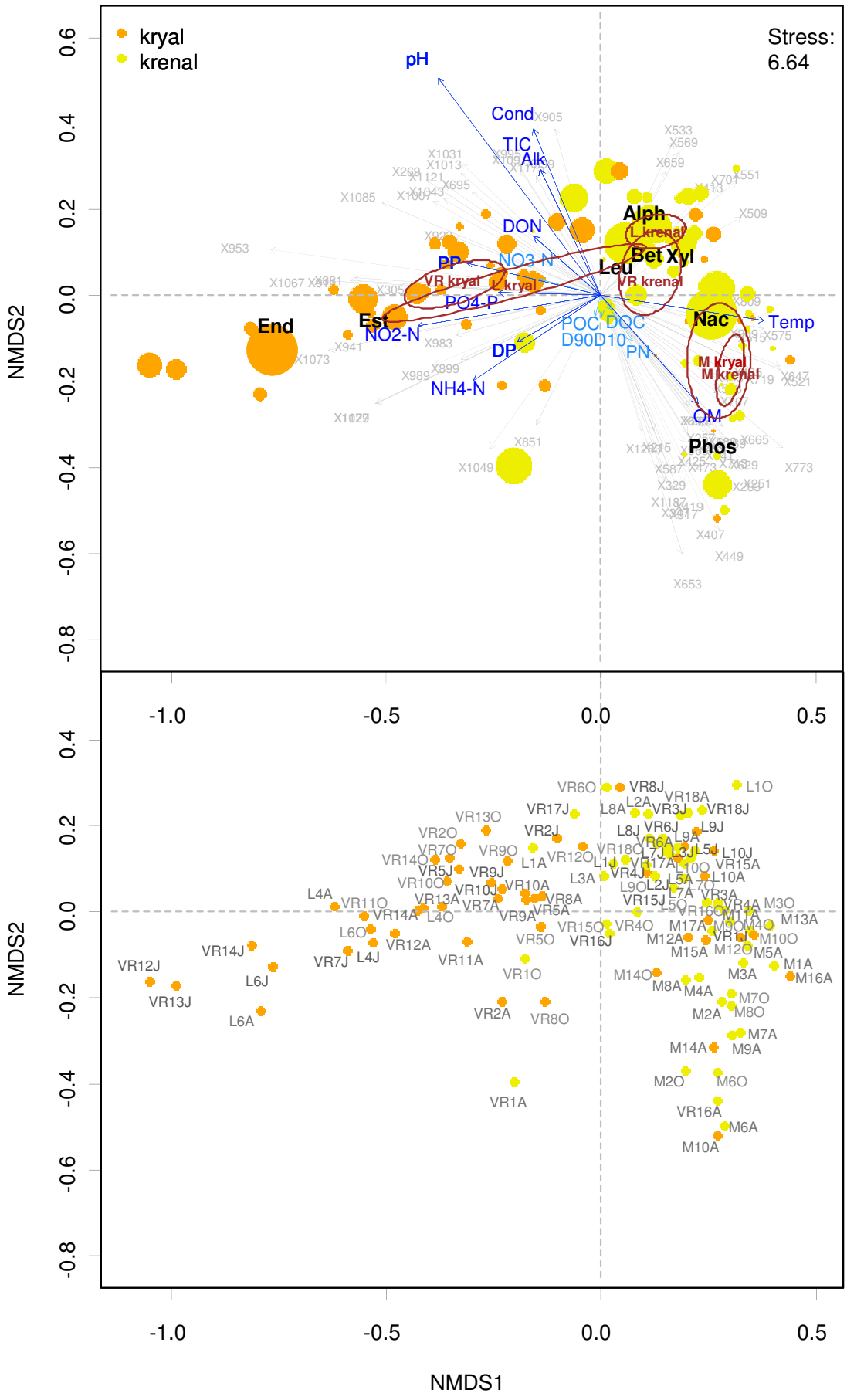
Appendix Figure 5a to 5c

NMDS of ARISA profiles with response surface as assessed by generalized additive models (GAM) for the measured physico-chemical variables. Each contour line is annotated with the specific value of the variable. The percentages of variances explained by variables are given and correspond to the values from appendix table 7.

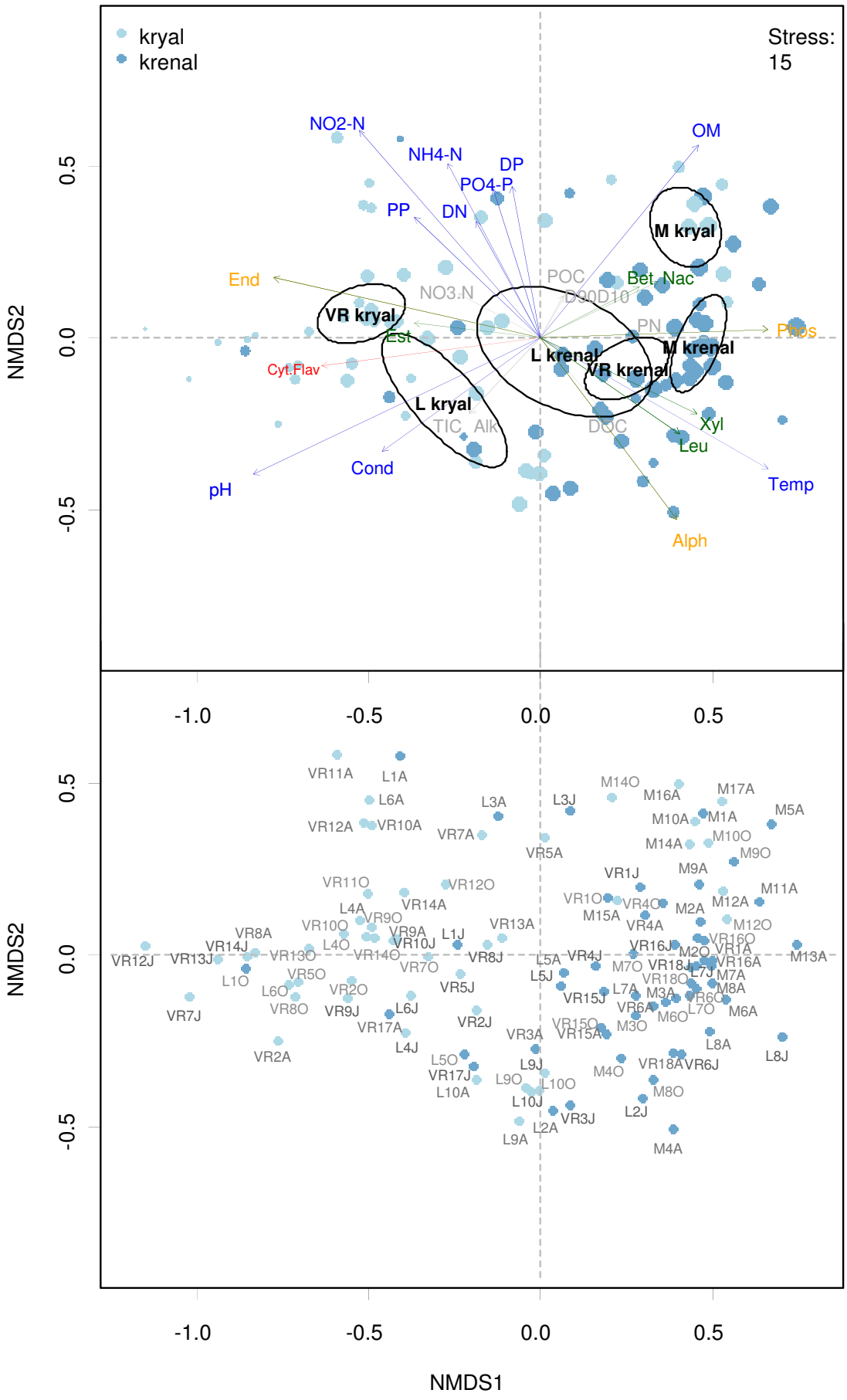
Appendix Figure 1



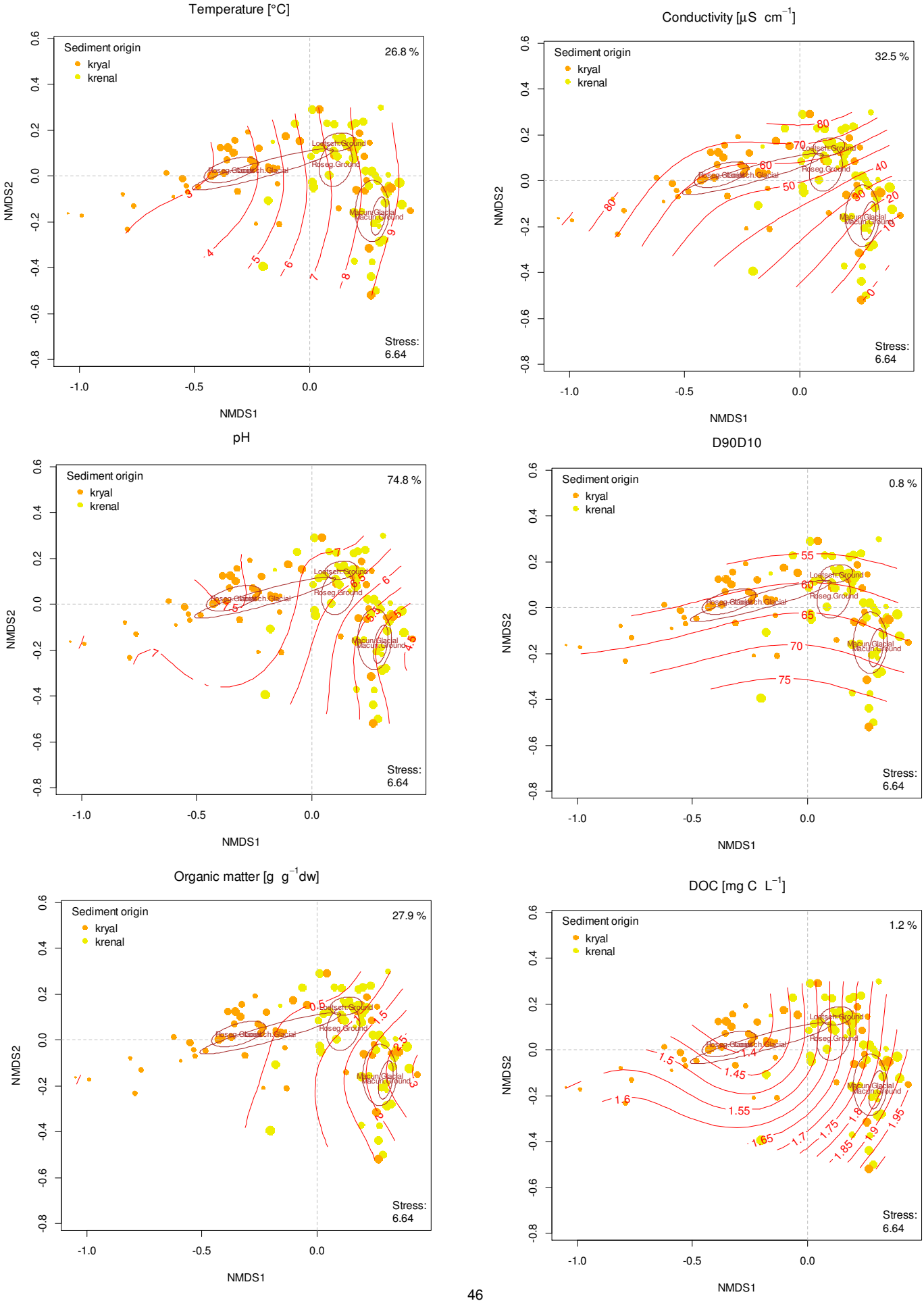
Appendix Figure 2



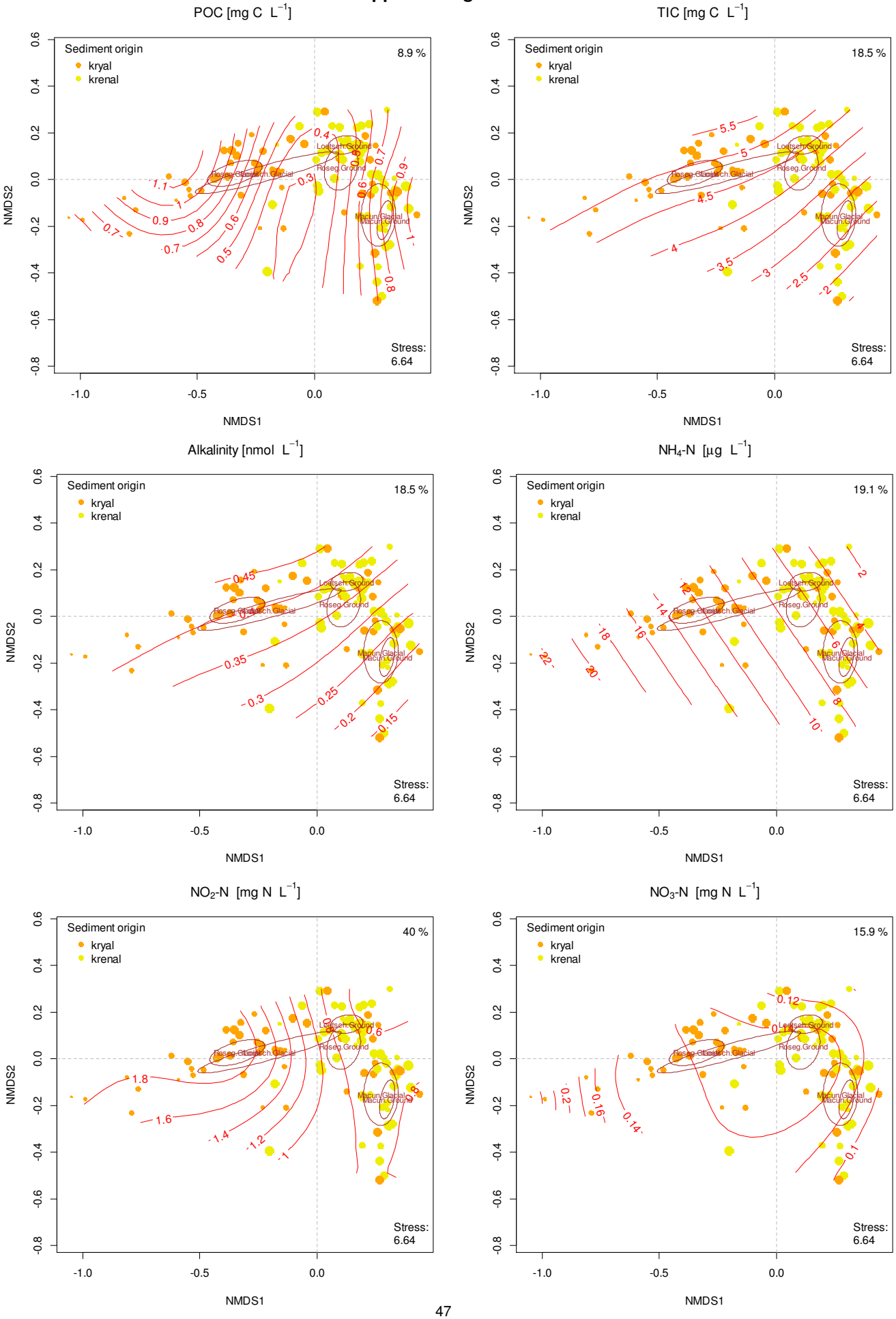
Appendix Figure 3



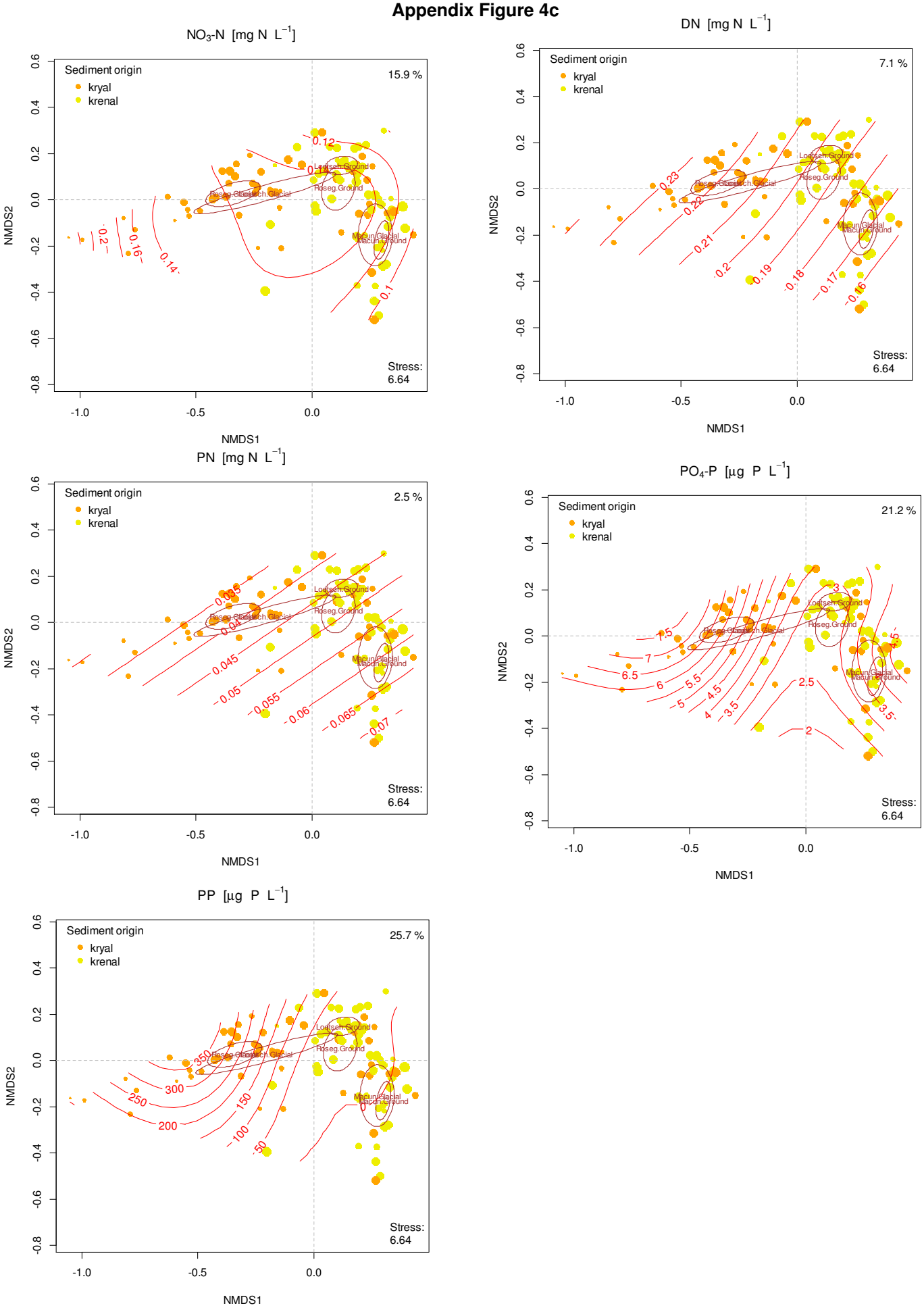
Appendix Figure 4a



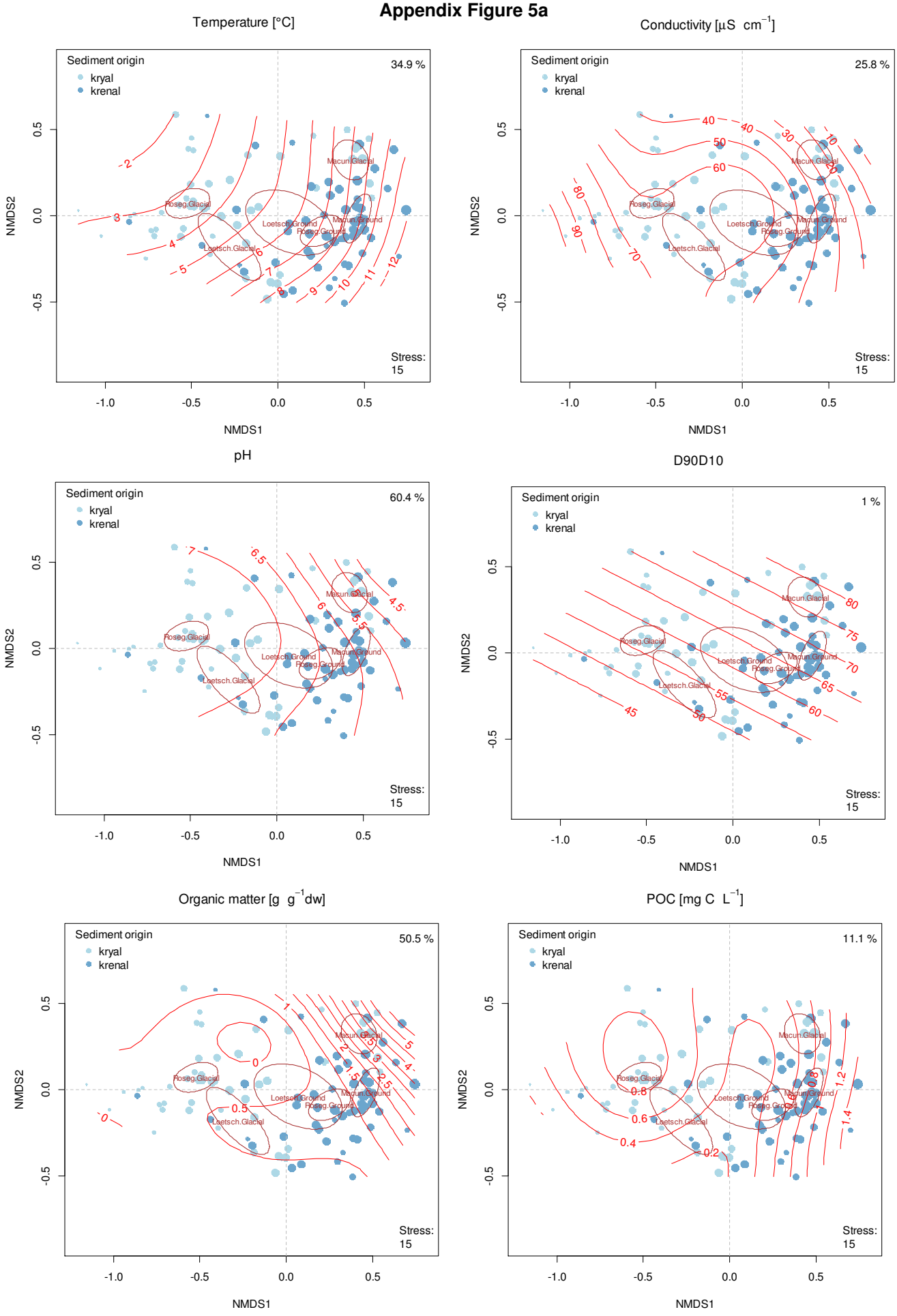
Appendix Figure 4b



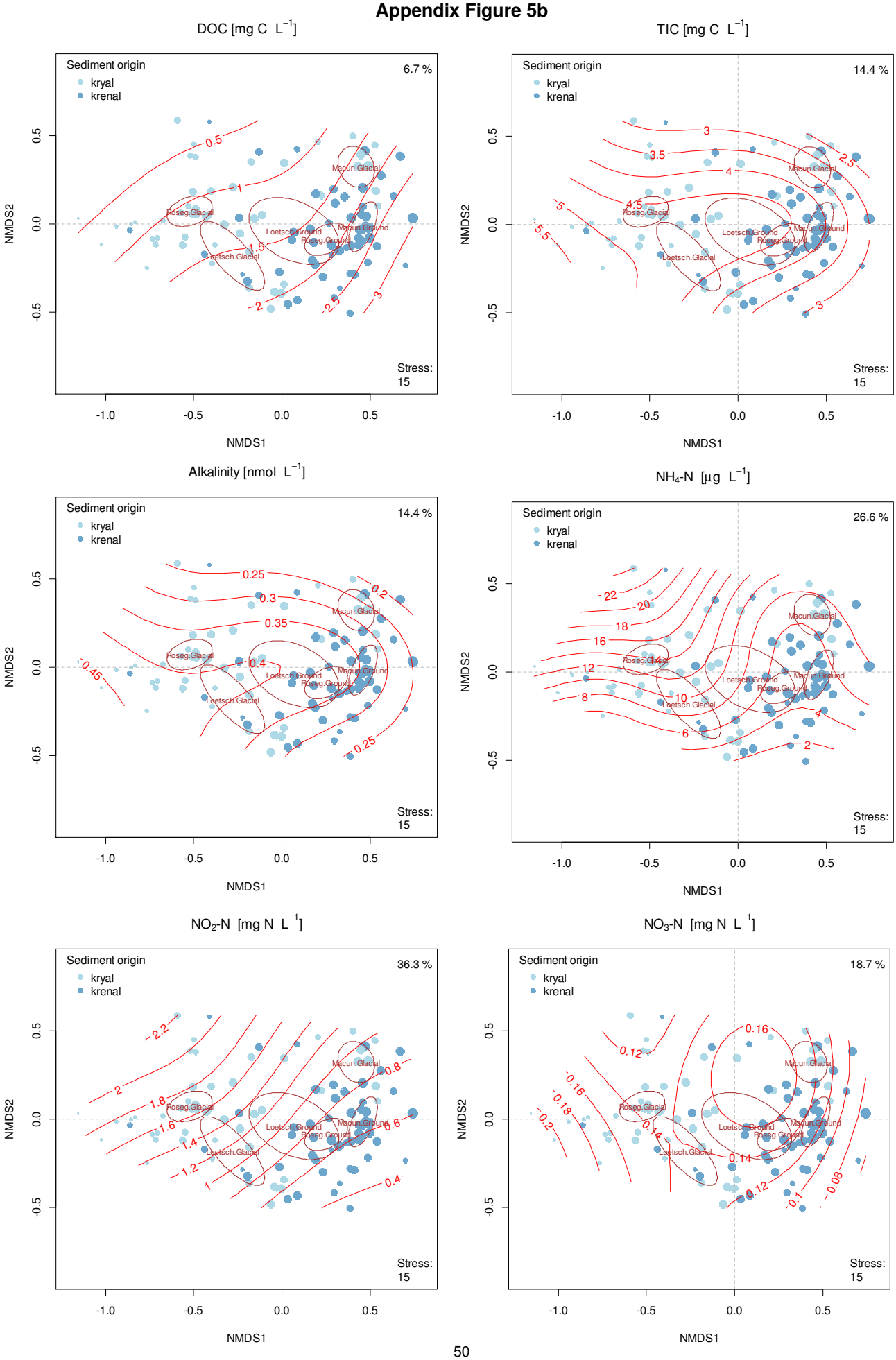
Appendix Figure 4c



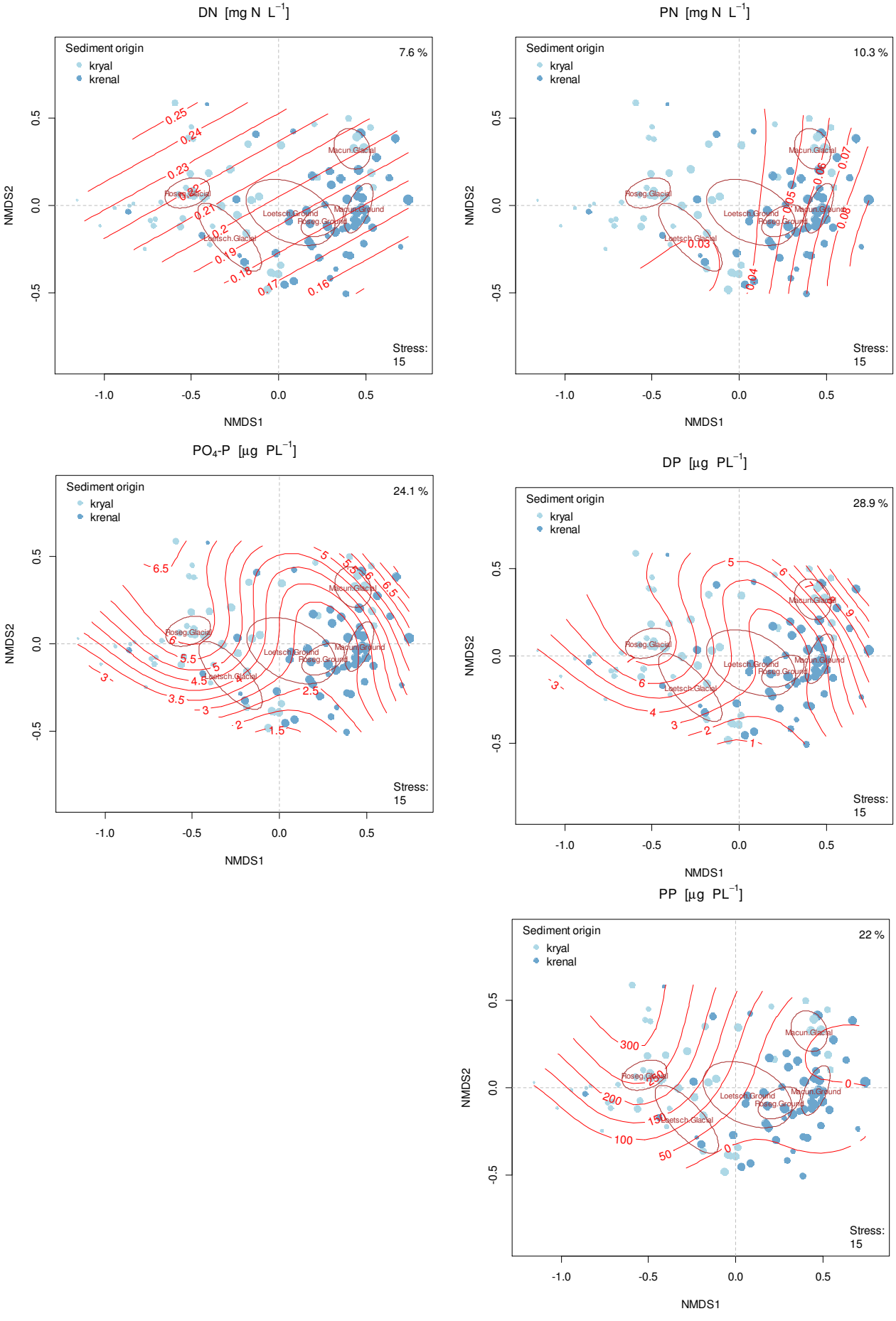
Appendix Figure 5a



Appendix Figure 5b



Appendix Figure 5c



Appendix Table 1 Physico-chemical parameters, given are the average ± standard deviation

Physico-chemical and microbial parameters

Catchment	Season	Stream type	Temperature [°C]	Conductivity [µS cm-1]	pH	D90D10	OM [g g-1dw]	DOC [mg C L-1]	POC [mg C L-1]	TIC [mg C L-1]	Alkalinity [mmol L-1]
Val Roseg	Summer	kryal	3.12 ± 2.16	25.12 ± 1.83	7.51 ± 0.34	71.99 ± 51.69	0.31 ± 0.10	0.77 ± 1.66	0.36 ± 0.10	2.78 ± 0.30	0.23 ± 0.02
		krenal	6.98 ± 1.96	44.20 ± 27.96	7.00 ± 0.38	111.26 ± 126.51	0.49 ± 0.22	0.39 ± 0.20	0.27 ± 0.17	5.12 ± 2.80	0.43 ± 0.23
	Winter	kryal	2.46 ± 1.73	58.97 ± 17.42	7.58 ± 0.41	48.67 ± 43.22	0.35 ± 0.05	1.47 ± 1.02	1.58 ± 2.60	5.79 ± 1.94	0.48 ± 0.16
		krenal	3.65 ± 0.55	72.57 ± 22.74	6.93 ± 0.30	36.48 ± 23.57	0.87 ± 0.84	1.00 ± 0.53	0.16 ± 0.10	7.58 ± 1.81	0.63 ± 0.15
	Spring	kryal	3.69 ± 2.27	102.91 ± 14.87	7.48 ± 0.32	56.12 ± 74.65	0.31 ± 0.09	0.37 ± 0.24	0.27 ± 0.10	5.60 ± 0.99	0.47 ± 0.08
		krenal	6.23 ± 2.64	78.54 ± 41.03	6.90 ± 0.38	156.26 ± 267.12	1.10 ± 1.20	1.69 ± 3.18	0.20 ± 0.05	6.51 ± 2.62	0.54 ± 0.22
Loetschental	Summer	kryal	6.20 ± 2.86	27.63 ± 6.99	6.79 ± 0.35	14.41 ± 3.02	0.33 ± 0.04	0.13 ± 0.04	0.42 ± 0.32	2.66 ± 0.57	0.22 ± 0.05
		krenal	13.67 ± 5.63	76.38 ± 28.97	6.51 ± 0.26	58.77 ± 65.82	0.74 ± 0.33	0.48 ± 0.34	0.27 ± 0.09	4.69 ± 1.54	0.39 ± 0.13
	Winter	kryal	5.00 ± 2.03	40.25 ± 11.44	6.40 ± 0.32	57.41 ± 75.49	0.88 ± 0.58	3.59 ± 3.87	0.44 ± 0.36	4.12 ± 0.75	0.34 ± 0.06
		krenal	6.43 ± 3.25	102.67 ± 87.36	6.53 ± 0.27	27.81 ± 15.83	0.65 ± 0.38	1.06 ± 0.61	1.89 ± 3.14	4.74 ± 1.06	0.39 ± 0.09
	Spring	kryal	5.40 ± 1.92	66.20 ± 27.84	7.05 ± 0.40	14.30 ± 2.88	0.14 ± 0.04	5.70 ± 5.58	0.34 ± 0.13	3.25 ± 1.00	0.27 ± 0.08
		krenal	9.43 ± 4.63	96.88 ± 48.09	6.53 ± 0.24	97.42 ± 122.04	0.51 ± 0.22	3.38 ± 5.64	0.61 ± 0.60	4.33 ± 1.93	0.36 ± 0.16
Macun	Summer	kryal	7.08 ± 5.13	6.45 ± 3.18	4.80 ± 0.24	63.71 ± 66.58	2.64 ± 2.74	0.45 ± 0.09	0.37 ± 0.26	2.29 ± 1.46	0.19 ± 0.12
		krenal	13.75 ± 3.01	7.20 ± 2.85	4.79 ± 0.35	48.10 ± 27.24	3.28 ± 3.72	1.23 ± 1.04	0.92 ± 1.44	2.13 ± 1.37	0.18 ± 0.11
	Winter	kryal	4.20 ± 0.14	12.00 ± 1.41	4.80 ± 0.09	24.24 ± 19.52	1.14 ± 0.06	1.08 ± 0.18	0.34 ± 0.28	0.83 ± 0.02	0.07 ± 0.00
		krenal	4.45 ± 0.90	9.04 ± 3.74	5.03 ± 0.25	44.26 ± 22.97	1.81 ± 1.47	4.98 ± 5.56	1.53 ± 1.12	1.16 ± 0.21	0.10 ± 0.02

Catchment	Season	Stream type	NH_4-N [µg L-1]	NO_2-N [µg N L-1]	NO_3-N [mg N L-1]	DN [mg N L-1]	PN [mg N L-1]	PO_4-P [µg P L-1]	DP [µg P L-1]	PP [µg P L-1]	Bacteria abundance
Val Roseg	Summer	kryal	22.12 ± 15.28	2.64 ± 0.40	<0.1 ± 0.00	<0.25 ± 0.00	0.03 ± 0.00	5.29 ± 2.41	5.71 ± 2.83	219.05 ± 81.59	2.01E+07 ± 1.44E+07
		krenal	<2.5 ± 0.00	0.58 ± 0.21	0.15 ± 0.07	<0.25 ± 0.00	0.02 ± 0.02	<2.5 ± 0.00	<2.5 ± 0.00	10.93 ± 14.28	1.20E+08 ± 1.34E+08
	Winter	kryal	7.35 ± 5.95	1.69 ± 1.54	<0.1 ± 0.00	0.22 ± 0.05	0.06 ± 0.06	7.94 ± 7.02	9.10 ± 8.79	454.72 ± 722.43	4.68E+06 ± 2.31E+06
		krenal	3.02 ± 1.27	<0.5 ± 0.00	0.15 ± 0.06	0.19 ± 0.07	0.01 ± 0.01	<2.5 ± 0.00	2.97 ± 1.14	1.68 ± 1.49	3.12E+08 ± 2.56E+08
	Spring	kryal	12.36 ± 7.31	1.73 ± 0.50	0.24 ± 0.12	<0.25 ± 0.00	0.04 ± 0.01	3.50 ± 1.65	4.57 ± 2.24	40.78 ± 37.04	5.72E+06 ± 4.86E+06
		krenal	<2.5 ± 0.00	<0.5 ± 0.00	0.19 ± 0.10	<0.25 ± 0.00	0.03 ± 0.02	<2.5 ± 0.00	<2.5 ± 0.00	0.79 ± 0.54	4.73E+08 ± 9.31E+08
Loetschental	Summer	kryal	6.80 ± 5.28	0.78 ± 0.32	0.08 ± 0.01	0.07 ± 0.03	0.02 ± 0.01	<2.5 ± 0.00	<2.5 ± 0.00	154.91 ± 165.75	1.11E+07 ± 6.00E+06
		krenal	7.27 ± 6.15	0.58 ± 0.20	0.06 ± 0.04	0.10 ± 0.09	0.02 ± 0.01	<2.5 ± 0.00	<2.5 ± 0.00	20.77 ± 19.74	5.46E+07 ± 6.80E+07
	Winter	kryal	<2.5 ± 0.00	<0.5 ± 0.00	<0.1 ± 0.00	0.11 ± 0.02	0.02 ± 0.01	2.53 ± 2.06	2.28 ± 2.12	62.68 ± 79.16	5.99E+06 ± 2.24E+06
		krenal	<2.5 ± 0.00	<0.5 ± 0.00	<0.1 ± 0.00	0.10 ± 0.04	0.17 ± 0.29	1.43 ± 0.45	1.37 ± 0.35	56.79 ± 96.08	7.72E+07 ± 7.44E+07
	Spring	kryal	20.30 ± 14.07	1.13 ± 0.61	<0.1 ± 0.00	0.24 ± 0.01	0.04 ± 0.01	7.05 ± 6.55	8.65 ± 9.65	41.60 ± 52.78	9.22E+06 ± 5.88E+06
		krenal	11.35 ± 8.92	0.80 ± 0.59	<0.1 ± 0.00	<0.25 ± 0.00	0.06 ± 0.05	4.58 ± 2.89	4.77 ± 3.23	14.26 ± 10.24	4.54E+07 ± 4.30E+07
Macun	Summer	kryal	5.22 ± 4.26	<0.5 ± 0.00	0.15 ± 0.04	0.16 ± 0.04	0.03 ± 0.01	6.18 ± 4.80	7.50 ± 5.31	2.45 ± 1.94	5.84E+08 ± 8.51E+08
		krenal	6.14 ± 5.06	<0.5 ± 0.00	0.05 ± 0.03	0.11 ± 0.05	0.07 ± 0.05	4.56 ± 3.20	6.18 ± 5.24	2.80 ± 2.96	1.14E+09 ± 1.46E+09
	Winter	kryal	<2.5 ± 0.00	<0.5 ± 0.00	0.32 ± 0.05	0.45 ± 0.02	0.06 ± 0.04	<2.5 ± 0.00	<2.5 ± 0.00	2.83 ± 3.30	1.30E+08 ± 3.42E+07
		krenal	11.06 ± 16.75	0.56 ± 0.18	<0.1 ± 0.00	0.18 ± 0.06	0.11 ± 0.07	<2.5 ± 0.00	3.74 ± 2.61	10.13 ± 14.79	1.85E+08 ± 1.21E+08

Appendix Table 2 Summary of ANOVA analysis for the eight measured enzymes, see text for enzyme abbreviation

		Function								
Source of variation	df	Alph	Bet	Xyl	Est	Nac	Leu	End	Phos	total activity
Catchment (C)	2	17.69***	11.13***	6.01*	85.93***	0.51	25.24***	65.86***	4.73*	32.51***
Watersystem (W)	1	28.87***	28.77***	43.64***	3.10	33.15***	19.87***	14.85***	48.07***	17.43***
Season (S)	2	1.35	2.08	1.71	1.38	0.85	1.29	1.51	1.21	1.45
C x W	2	2.37	3.33*	4.98**	1.96	5.66**	1.94	2.84	6.02**	1.60
C x S	3	0.68	1.94	2.72*	1.15	4.61**	2.04	0.41	1.94	2.18
W x S	2	0.19	0.06	0.17	1.00	1.84	2.11	0.76	0.80	0.92
C x W x S	3	1.56	3.64*	2.94*	0.29	1.26	1.24	0.55	1.16	1.67

* P<0.05

** P<0.01

*** P<0.001

Appendix Table 3 Enzymatic activities (average±SD), see main text for abbreviations

			Enzymatic activities [n mol substrate g^{-1} h^{-1}]							
Catchment	Season	Stream type	Alph	Bet	Xyl	Nac	Est	Leu	End	Phos
Val Roseg	Summer	kryal	140.05 ± 247.78	451.96 ± 975.96	31.93 ± 56.48	154.99 ± 278.28	1223.42 ± 1775.94	1954.70 ± 1677.38	889.43 ± 977.47	823.11 ± 1268.83
		krenal	31.66 ± 30.46	139.66 ± 119.41	17.19 ± 14.00	49.26 ± 46.11	263.82 ± 320.36	1112.38 ± 1027.14	254.85 ± 392.54	846.22 ± 1937.84
	Winter	kryal	46.68 ± 66.94	135.86 ± 124.61	11.00 ± 7.64	24.17 ± 26.16	635.42 ± 1112.56	2583.17 ± 2055.72	551.36 ± 790.16	149.77 ± 155.01
		krenal	79.79 ± 95.86	171.09 ± 53.18	21.11 ± 30.82	35.08 ± 47.75	192.91 ± 122.08	1216.68 ± 1016.07	201.56 ± 152.75	319.44 ± 347.98
	Spring	kryal	93.97 ± 121.05	1056.30 ± 2819.56	75.38 ± 195.53	277.40 ± 742.16	678.79 ± 796.08	2039.96 ± 3741.62	956.65 ± 1405.94	684.42 ± 1716.88
		krenal	63.87 ± 51.31	155.23 ± 144.07	16.59 ± 15.68	30.07 ± 34.54	629.17 ± 766.15	3281.35 ± 6204.47	797.42 ± 916.61	198.16 ± 175.68
Loetschental	Summer	kryal	9.62 ± 6.43	14.56 ± 15.53	2.52 ± 2.24	11.41 ± 19.24	371.30 ± 523.01	215.43 ± 90.65	357.01 ± 412.59	45.31 ± 12.96
		krenal	71.58 ± 68.39	153.83 ± 120.23	26.32 ± 30.07	34.73 ± 41.15	109.92 ± 98.30	524.60 ± 392.76	64.03 ± 66.92	153.34 ± 126.01
	Winter	kryal	7.70 ± 4.02	14.59 ± 6.46	3.38 ± 2.19	5.93 ± 5.24	219.38 ± 263.20	191.66 ± 92.50	168.47 ± 204.89	19.83 ± 7.79
		krenal	91.32 ± 74.85	215.83 ± 227.00	38.00 ± 39.87	104.15 ± 71.68	104.10 ± 86.64	1841.93 ± 2358.75	47.08 ± 40.37	325.85 ± 328.77
	Spring	kryal	44.96 ± 24.29	197.70 ± 173.35	24.48 ± 14.46	73.88 ± 103.59	4307.61 ± 7921.58	1201.03 ± 669.86	2967.09 ± 5375.57	139.74 ± 67.23
		krenal	114.60 ± 145.52	385.09 ± 571.94	57.60 ± 76.13	124.53 ± 157.96	205.81 ± 281.25	1696.96 ± 2981.65	109.16 ± 143.18	269.46 ± 307.60
Macun	Summer	kryal	16.07 ± 14.30	83.32 ± 66.18	12.21 ± 11.07	54.66 ± 58.92	5.97 ± 3.59	188.28 ± 164.70	6.81 ± 3.86	246.64 ± 132.72
		krenal	11.91 ± 16.49	65.43 ± 74.42	8.42 ± 7.25	18.59 ± 9.72	78.10 ± 233.95	445.19 ± 522.79	54.30 ± 169.79	270.28 ± 228.30
	Winter	kryal	7.96 ± 1.46	37.44 ± 0.94	4.65 ± 0.69	20.02 ± 6.91	4.64 ± 0.63	185.96 ± 75.84	0.08 ± 0.07	99.04 ± 8.25
		krenal	24.92 ± 37.78	109.64 ± 174.89	8.47 ± 10.76	50.30 ± 53.57	12.83 ± 4.90	523.21 ± 415.67	2.76 ± 2.81	416.78 ± 263.70

Appendix Table 4 F-Values of pairwise PERMANOVA comparisons of community structure and enzymatic activities. Groups are split by catchments, water source and season, dF_{tot} are given in parentheses

		Catchment	***Macun***				***Val Roseg***						***Loetschental***					
		Season	*Summer*		*Winter*		*Summer*		*Winter*		*Spring*		*Summer*		*Winter*		*Spring*	
Catchment	Season	Water	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*	*krenal*	*kryal*
Macun	*Summer*	*krenal*	**ARISA**	(17)0.89	(17)0.99	(14)0.82	(19)3.11*	(21)41.96***	(17)9.13***	(21)31.67***	(19)10.48***	(20)27.84***	(17)12.57***	(15)8.98***	(14)5.38**	(15)9.84**	(17)2.79***	(15)7.85***
		kryal	(17)1.41*		(11)1.55	(8)0.68	(13)2.18	(15)29.24***	(11)6.7**	(15)19.41***	(13)7.08**	(14)14.72**	(11)8.08**	(9)4.86**	(8)3.71*	(9)6.03**	(11)8.56**	(9)4.26**
	Winter	*krenal*	(17)1.75*	(11)1.97**		(8)1.04	(13)2.05	(15)39.43***	(11)5.89**	(15)22.74***	(13)8.30**	(14)17.29***	(11)13.13**	(9)5.75**	(8)4.75*	(9)7.75**	(11)16.44**	(9)5.37*
		kryal	(14)1.52*	(8)0.77	(8)1.79*		(10)0.85	(12)25.02**	(8)3.91*	(12)11.48**	(10)3.88*	(11)8.16*	(8)5.90**	(6)2.52	(5)3.33	(6)3.75	(8)9.64*	(6)3.54*
Val Roseg	*Summer*	*krenal*	(19)2.54***	(13)2.04***	(13)2.23**	(10)1.75*		(17)14.55***	(13)2.48*	(17)11.51***	(15)1.17	(16)11.17**	(13)1.95	(11)2.62	(10)1.09	(11)2.50*	(13)2.12	(11)2.52
		kryal	(21)7.41***	(15)4.50***	(15)5.16**	(12)3.43**	(17)3.98***		(15)9.38***	(19)2.72*	(17)21.34***	(18)2.27	(15)20.42**	(13)0.60	(12)8.71**	(13)0.87	(15)35.46**	(13)0.094
	Winter	*krenal*	(17)2.37***	(11)1.90**	(11)2.20**	(8)2.04*	(13)0.63	(15)4.63***		(15)5.85**	(13)2.85*	(14)7.54*	(11)4.24**	(9)1.95	(8)2.33	(9)1.57	(11)5.90**	(9)1.59
		kryal	(21)11.75***	(15)7.91***	(15)8.56***	(12)6.51**	(17)6.47***	(19)2.71**	(15)7.87***		(17)12.48***	(18)2.91	(15)10.10***	(13)1.29	(12)9.28**	(13)1.23	(15)15.99***	(13)0.80
	Spring	*krenal*	(19)2.78***	(13)2.29***	(13)2.26***	(10)1.96**	(15)0.54	(17)4.59***	(13)0.78	(17)7.44***		(16)12.92**	(13)1.01	(11)3.34	(10)0.82	(11)3.00*	(13)0.80	(11)2.83
		kryal	(20)9.14***	(14)5.98***	(14)6.25**	(11)4.86**	(16)4.61***	(18)2.77**	(14)5.71***	(18)1.56	(16)5.32***		(14)8.58**	(12)0.89	(11)6.87*	(12)1.86	(14)11.58**	(12)1.09
Loetschental	*Summer*	*krenal*	(17)2.47***	(11)1.68**	(11)1.98**	(8)1.52*	(13)0.90	(15)2.87***	(11)1.32	(15)4.76***	(13)1.13	(14)3.48**		(9)2.10	(8)2.32	(9)2.54	(11)1.14	(9)2.01
		kryal	(15)4.17***	(9)2.84**	(9)3.19**	(6)2.54*	(11)1.94*	(13)1.85*	(9)2.88**	(13)3.19**	(11)2.40***	(12)2.53*	(9)1.69*		(6)2.00	(7)0.18	(9)3.16	(7)0.13
	Winter	*krenal*	(14)2.41**	(8)1.73*	(8)1.81*	(5)1.55	(10)1.19	(12)1.65	(8)1.52*	(12)2.44*	(10)1.45*	(11)1.85	(8)0.83	(6)1.31		(6)2.44	(8)1.80	(6)1.71
		kryal	(15)4.60***	(9)3.30**	(9)3.72**	(6)3.06*	(11)2.28*	(13)2.87**	(9)3.20**	(13)3.32**	(11)2.59***	(12)2.62*	(9)2.11*	(7)0.61	(6)1.58		(9)4.29*	(7)0.08
	Spring	*krenal*	(17)1.98**	(11)1.45*	(1)1.71*	(8)1.42	(13)0.91	(15)3.37**	(11)1.20	(15)6.11***	(13)1.07	(14)4.25***	(11)0.56	(9)1.90*	(8)0.75	(9)2.61**		(9)2.84
		kryal	(15)4.27**	(9)3.12***	(9)3.24**	(6)3.54*	(11)2.24**	(13)3.15**	(9)3.5**	(13)4.86***	(11)2.52***	(12)3.36**	(9)2.01*	(7)0.82	(6)1.43	(7)1.06	(9)2.03*	**Enzymes**

* P<0.05
** P<0.01
*** P<0.001

Appendix Table 5 Model parameters of AIC selected multiple linear regression between enzymatic activity and physico-chemical parameters and its relative importance metrics. Models are based on enzyme activities standardized to OM

			Multiple linear regression				Relative importance metrics	
Enzyme	AIC modell parameters	dF	Residual SE	multiple R^2adj	F-stat	P-Value	Total responce variance	Proportion explained by model
Alph	6	98	1.008	0.441	14.67	<0.001	1.818	0.473
Bet	7	97	1.003	0.464	13.86	<0.001	1.877	0.501
Xyl	7	97	0.929	0.376	9.941	<0.001	1.384	0.418
Nac	6	98	1.238	0.215	5.754	<0.001	1.95	0.26
Est	11	93	0.896	0.777	33.91	<0.001	3.597	0.805
Leu	7	97	1.072	0.461	11.84	<0.001	1.987	0.461
End	7	97	1.168	0.784	50.28	<0.001	5.89	0.784
Phos	10	94	1.071	0.401	7.975	<0.001	1.916	0.459

Appendix Table 6 Left side: explained deviance (Dev) of the response surface (generalized additive models) of the environmental variables and fitted factors with their squared correlation coefficients (r^2) on the EF NMDS. **Right side**: Canonical coefficients from the of forward selected physico-chemical parameters incorporated into the global RDA model explaining EF. Given are the first two constraint axes. Explained variations of the constraint axes are based on unadjusted R^2.

	NMDS				RDA canonical coefficients	
Variables	dF	F	Dev	P	RDA1 (48.90%)	RDA2 (2.96%)
Temperature	5.21	6.55	26.8%	<0.001		
Conductivity	6.22	7.22	32.5%	<0.001	-0.04	-0.03
pH	8.64	32.55	74.8%	<0.001	-0.17	-0.45
D90D10	2.45	0.17	0.8%	0.88		
OM	7.17	4.69	27.9%	<0.001		
DOC	2.84	0.23	1.2%	0.87		
POC	6.25	1.07	8.9%	0.39	0.05	-0.15
TIC	4.20	5.14	18.5%	<0.001		
Alkalinity	4.24	5.06	18.5%	<0.001		
NH_4-N	2.00	12.04	19.1%	<0.001		
NO_2-N	7.45	8.02	40.0%	<0.001		
NO_3-N	7.45	1.72	15.9%	0.11		
DN	2.58	3.05	7.1%	<0.05		
PN	2.00	1.30	2.5%	0.28		
PO_4-P	7.16	3.18	21.2%	<0.01		
DP	6.88	2.78	18.7%	<0.05	-0.03	-0.09
PP	6.96	4.39	25.7%	<0.001	-0.03	0.07
Factors			r^2	P		
Catchment			0.31	<0.001		
Season			0.07	<0.05		
Water			0.26	<0.001		

Appendix Table 7 Left side: explained deviance (Dev) of the response surface (generalized additive models) of the environmental variables and fitted factors with their squared correlation coefficients (r^2) on the BCC NMDS. **Right side**: Canonical coefficients from the of forward selected physico-chemical parameters incorporated into the global RDA model explaining BCC. Given are the first two constraint axes. Explained variations of the constraint axes are based on unadjusted R^2.

	NMDS				RDA canonical coefficients	
Variables	dF	F	Dev	P	RDA1 (14.6%)	RDA2 (3.8%)
Temperature	6.06	8.22	34.9%	<0.001	0.04	-0.07
Conductivity	7.28	3.95	25.8%	<0.001	-0.03	-0.02
pH	8.29	17.21	60.4%	<0.001	-0.05	-0.42
D90D10	2.00	0.50	1.0%	0.61	0.01	0.01
OM	8.58	10.74	50.5%	<0.001	0.14	0.30
DOC	4.04	1.23	6.7%	0.30		
POC	6.81	1.34	11.1%	0.24	0.06	-0.04
TIC	6.90	1.93	14.4%	0.07		
Alkalinity	6.94	1.92	14.4%	0.08		
NH_4-N	7.34	4.22	26.6%	<0.001		
NO_2-N	5.62	9.59	36.3%	<0.001	-0.08	0.13
NO_3-N	7.73	2.30	18.7%	<0.05	0.09	-0.08
DN	2.00	4.21	7.6%	<0.05		
PN	5.52	1.65	10.3%	0.15		
PO_4-P	7.86	3.05	24.1%	<0.01		
DP	8.30	3.82	28.9%	<0.001		
PP	6.82	3.50	22.0%	<0.01	-0.02	0.01
Factors			r^2	P		
Catchment			0.27	<0.001		
Season			0.06	<0.05		
Water			0.30	<0.001		

Paper 2

Structure and Function of Bacterial Communities within Alpine Floodplains: A Hydrological Point of View

Structure and Function of Bacterial Communities within Alpine Floodplains: A Hydrological Point of View

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Abstract

Microbial community assembly and dynamics are driven by coupled and differently contributing mechanisms such as local habitat characteristics, dispersal rates and species interactions. Little is known about the importance of hydrologic-mediated physico-chemical and biotic connectivity between floodplain habitats driving bacterial community composition and functioning. We studied bacterial communities and their associated functions (enzyme activities) along hydrologic flow paths within two alpine floodplains differing in landscape structure. We installed piezometers within the hyporheic and riparian zones within each floodplain, using them as incubation chambers for bacteria. One site was investigated during three different hydrological periods to include the potential effects of altered hydrologic connectivity. We used spatial models to gain insight of the importance of directed hydrological and non-directed spatially-mediated mechanisms on bacterial assemblages. Our results suggest a strong influence (up to 40% explained variation) of hydrological connectivity on bacterial functioning corresponding with gradual changes along each flow path. Community assembly was less influenced by the hydrologic linkage, indicating a high degree of functional plasticity within alpine bacterial communities. The distinction of hyporheic and riparian zone communities appeared dependent on the landscape structuring within each floodplain. An emerging importance of non-directed spatial processes drove bacterial assembly and function during hydrologically less-active periods. Our results demonstrate the importance of hydrologic conditions within alpine floodplains driving bacterial (ecosystem) functioning and community dynamics. The impact of changing hydrology, thus landscape connectivity, should be considered in future models predicting ecosystem services of floodplains, particularly the rapid changes now occurring in alpine landscapes.

Introduction

Alpine floodplains consist of directional hydrologic networks linking different floodplain habitats. Streams, lakes, riparian-, groundwater- and hyporheic zones are such landscape structures present in floodplains that are connected by surface and subsurface water flow. Depending on the specific structuring and hydrologic interactions of this network, e.g. the relative spatial position of landscape structures along hydrological flow paths or the rate of water flow, different effects on physico-chemical and biological characteristics can be expected that form distinct habitat patches (Tockner *et al.,* 1997, Kling *et al.,* 2000, Riera *et al.,* 2000, Brunke *et al.,* 2003, Robinson *et al.,* 2007, Robinson and Matthaei 2007). Although bacteria play a key role in ecosystem functioning due to their ubiquity and integral role in various functional processes, there is still little understanding of the underlying mechanisms that determine bacterial community composition and respective functioning within such habitat patches.

The metacommunity framework invokes underlying mechanisms to explain species turnover within space and time in the context of environmental, dispersal, and stochastic mechanisms (Leibold *et al.,* 2004). Within the metacommunity framework, community composition can vary among habitats due to local environmental factors favoring more adapted and competitive species (species sorting) (Logue and Lindström 2010). Furthermore, ecosystem function (e.g. enzymatic activity) can be regulated by local environmental conditions such as the quality of dissolved organic carbon (Ayuso *et al.,* 2011). In landscapes that contain aquatic compartments, such as alpine floodplains, hydrology strongly influences ecosystem characteristics and thus ecosystem function. For instance, the rate of sediment respiration and enzymatic activity can be influenced by the water saturation state of sediments and by water temperature (Doering *et al.,* 2011, Brockett *et al.,* 2012).

Hydrodynamics play an integral role for bacterial community assembly in benthic biofilms (Besemer *et al.,* 2009a). At the larger scale, floodplain compartments are hydrologically interconnected and local hydrodynamics substantially dictate connectivity between hyporheic sediments and riparian patches due to different water in- and exfiltration rates. The strength of spatial hydrologic connectivity such as rates of water flow dictate residence times and chemical processes; e.g. nutrients that are transported through the hydrological network (Argerich *et al.,* 2008). The morphology of a floodplain and its compartments thus directly influence hydrological exchange properties and potential functioning, e.g. nutrient uptake rates (Argerich *et al.,* 2008, Buffington and Tonina 2009). The distribution of populations within habitat patches can therefore also be influenced by the migration of chemical compounds along hydrological flow paths, which then partially determine local environmental conditions. Importantly, such successive changes in chemical composition also drive functions performed by bacterial assemblages (Kolehmainen *et al.,* 2009).

The specific characteristics and degree of interaction of bacteria present in an ecosystem can influence community assembly and persistence (Lindström and Langenheder 2011). Depending on genotypic plasticity and/or functional redundancy apparent in a bacterial assemblage, changing environmental constraints may have different effects on a community compositional trajectory. I.e. Community may not change if plasticity and functional redundancy is high. In contrast a community could develop towards functionally most competitive species when plasticity is not apparent and redundancy is low. For instance, Findlay et al. (2003) showed that hyporheic bacterial structure and metabolic functions changed due to altered dissolved organic matter composition thus indicating minor functional plasticity and/or low redundancy. In addition to this finding, nitrogen amended hyporheic microbial communities changed their enzymatic activity patterns but not their community structure indicating high functional plasticity (Findlay and Sinsabaugh 2003). These findings suggest that species sorting depends on the specific environmental constraint in combination with the degree of functional plasticity and redundancy of a specific trait apparent in a bacterial community.

The patch-dynamics and the neutral models of the metacommunity framework conceptualize on how environmental heterogeneity can play a role in species trait differences that drive bacterial community composition. Patch dynamics refers to a community assembly mediated by regional colonization and extinction events that are dependent on the trade-off between specific species traits (Yu and Wilson 2001). For example, invasive species that disperse easily can out-compete less adapted species in a habitat patch, whereas good competitors could resist such invasions. In the neutral model, all species share the same traits and habitat patches show homogeneity, and the metacommunity framework suggests a stochastic community assembly. This mechanism would be driven by arbitrary dispersal, colonization success and evolutionary mechanisms such as speciation and extinction (Volkov *et al.,* 2003).

Drift also affects species assemblage mechanisms in flow-mediated environments. According to the metacommunity framework, dispersal from spatially-linked local habitats can influence the local species pool thus is a regional factor (Crump *et al.,*

2007). Bacteria can be distributed actively or passively along flow paths and thus reach downstream habitat patches (Cousin 2009). The strength in flow and the dispersal characteristics of bacteria ultimately determine the impact of such a mass effect on bacterial assembly in local habitat patches. Lakes with short retention time have been shown to be greatly influenced by mass effects, whereas lakes with long retention time mainly were characterized by species sorting effects affecting bacterial assembly (Lindström and Bergström 2004, Lindström *et al.,* 2006).

It is likely that a continuum between local and regional factors influences local bacterial communities (Logue *et al.,* 2011). Importantly, temporal fluctuations in environmental factors within habitat patches not only constrain bacteria assembly but also add a historical component to environmental effects. I.e. colonizing bacteria can gain a general evolutionary advantage or have nutritional advantages through the efficient use and "monopolization" of local resources (Loeuille and Leibold 2008, Urban and De Meester 2009). Even ecosystem functioning can be altered by historical colonization events (Fukami and Morin 2003). Ultimately, the relative strength and temporal variability of these mechanisms will influence community composition and beta diversity between habitat patches (Leibold *et al.,* 2004, Langenheder *et al.,* 2011, Langenheder and Székely 2011).

Alpine floodplains consist of different hydrologically-connected landscape compartments that each show distinct fluctuations in physico-chemical characteristics and variable hydrologic connectivity among each other over time (Ward *et al.,* 1998). We conducted this study in a glaciated alpine catchment to assess bacterial community assembly and functioning in the context of hydrological and spatial connectivity within and between habitat patches; i.e., between hyporheic sediments and riparian habitats, along hydrological flow paths. We installed a set of piezometers in the streams and the adjacent riparian zone in two floodplains within the catchment to assess community composition and potential enzyme activities of bacterial assemblages colonizing each piezometer. Different hydrological periods were examined at one site to gain insight on the importance of temporal changes in hydrological linkages on bacterial communities and functioning. The second site was used as a contrasting system to the first due to the presence of a pro-glacial lake, differences in riparian vegetation and general landscape features that could influence hydrological conditions. The results are discussed in the context of the metacommunity framework.

Material and methods

Site description and sampling

Study floodplains were associated with small tributary streams in Val Roseg catchment, a glaciated alpine valley in the upper Engadin, Switzerland (Figure 1). Around 30% of the valley is glaciated (BAFU 2010). We chose two floodplain sites that contrasted with regard to the local tributaries, relative location within the valley and their hydrological situation. One floodplain was associated with Remo creek (Site RC, N46°25''13', E9°51''41'), a perennial groundwater-fed (krenal) tributary draining into the Roseg River. The contribution of snowmelt water to the stream occurs mainly during spring. Elevation of this floodplain site is ca. 2044 m a.s.l. The floodplain is usually snow covered from November until May. The riparian zone becomes saturated in spring due to nascent snow melt. Towards summer the system dries and becomes wetted again when rain events and first snowfall starts in autumn. The riparian zone can be characterized as grassland with adjacent coniferous forests (Figure 1) (Zah and Uehlinger 2001).

The second floodplain was situated south of the moraine of the Tschierva glacier and harbors Simone Creek (site SC, N46°24''19', E9°51''18', Figure 1). The average elevation of this floodplain is ca. 2161 m a.s.l. In spring, the hydrology of the floodplain is mainly driven by snowmelt from snowpacks on the side moraine and the adjacent peaks. Minor flow during summer is from spring water and most floodplain channels run dry before autumn. In autumn, there is again increased water flow due to rain and snowfall events. The creek flows into Lake Roseg, a pro-glacial lake of the Roseg glacier complex. During hydrologic peak flows, the lower floodplain is wet and almost swamp-like. Soils near the Lake are essentially humid most of the year. Riparian vegetation consists of shrubs (*Salix* spp.) and grass.

Polyvinyl-chloride piezometers (86 cm long, 5 cm inner diameter, 5 mm pores) were installed in October 2008 at RC and in July 2009 at SC. The piezometers were fixed into the in-stream sediment and riparian soils as depicted in Figure 1. The geographical position of each piezometer was recorded with a geographic positioning system (Leica GPS 1200+) and a total station. Water potentials within each piezometer were measured on several dates in 2009 and 2010. Inverse distance weighted interpolation of water potentials provided insight into potential water flow directions within the floodplains during the study period (Appendix Figure 1) (Cressie 1993).

Sterilized 1-mm glass beads (Braun B. Biotech) were packed into individual 0.3- mm mesh nylon bags and placed at the bottom of each piezometer for colonization by bacteria. The bags with beads were harvested at site RC in June after 4 weeks of incubation, July (3 week incubation), and October (8 week incubation) 2010 at site RC and SC. Collected bags were transported in sterile Falcon tubes on ice to the laboratory and stored at -20°C until further processing.

Bacterial community fingerprinting

Bacterial community structure was assessed by automated ribosomal intergenic spacer amplification (ARISA). The PowerSoil DNA isolation Kit (MoBio, Carlsbad) was used to extract DNA from the incubated glass beads following manufacturer's instructions. DNA concentrations of extracts were below the detection limit (NanoDrop, Thermo Scientific). DNA was amplified using the fluorescein (6-FAM) labeled universal forward primer 1406f-6FAM (16S rRNA gene, 5'-FAM-TGYACACACCGCCCGT-3', Y=T,C) and the bacteria specific reverse primer 23Sr (5'-GGGTTBCCCCATTCRG-3', B=G,T,C, R=G,A) (Yannarell *et al.,* 2003). PCR was performed using a TProfessionalthermocycler (Biometra GmbH, Göttingen) in a final reaction volume of 25 µl with a mix of 1x GoTaq®Flexi buffer, 3 mM $MgCl_2$, 0.25 mM each of dNTP, 0.05 U μl^{-1} of GoTaq®Flexi DNA Polymerase (Promega, Switzerland), 0.25 mg ml^{-1} bovine serum albumin (Sigma-Aldrich, Switzerland), 0.4 µM of each primer (Microsynth, Switzerland), and 1 µl of template DNA (~ 1 ng). The reaction mix was initially denatured for 2 min at 94°C, followed by primer annealing at 55°C for 35 s and extension of 2 min at 72°C. Subsequently, 29 cycles with denaturation for 35 s and annealing and extension as above were performed, followed by a final extension of 2 min at 72°C. PCR products were subjected to electrophoresis on a 2% agarose gel with a 100 bp ladder (Promega, Switzerland) and stained with ethidium bromide to verify successful amplification. ARISA fragment analysis was performed as described in Bürgmann *et al*. (2011). A 1-µl aliquot of PCR product was mixed with 9 µl HiDi formamide and 0.5 µl Liz 1200 size standard (Applied Biosystems, Switzerland). Denaturation was performed on a PCR thermocycler for 3 min at 95°C and followed by capillary electrophoresis a 3130XL Capillary Genetic Analyzer (Applied Biosystems, Switzerland) equipped with a 50 cm capillary using POP-7 polymer. ARISA fragments were analyzed with the Southern size-calling method with a background cut-off level of 50 fluorescence units. Binning of peaks was done with automatic and interactive binning R scripts (Ramette 2009) leading to relative fluorescence intensity of peaks between 200 and 1400 bp.

Enzyme assays

Eight enzymes were tested for their potential activity using Methylumbelliferone (MUF)-labeled substrate analogues (Sigma-Aldrich CO). Enzymes which degrade polysaccharides were tested using 4-MUF-α-D-glucoside for Alpha glucosidase (Alph), 4-MUF-β-D-glucoside for Beta Glucosidase (Bet) and 4-MUF-β-D-xylopyranoide for β-xylosidase (Xyl). 4-MUF-N-acetyl-β-D-glucosaminide was used to assess hydrolysis by N-acetyl-glucosaminidase (Nac) (Sinsabaugh *et al.,* 2008). Esterase (Est) activity was measured with 4-MUF-acetate (Arpigny and Jaeger 1999). Leucine aminopeptidase (Leu) and endopeptidase (Epep) activity were measured using L-leucine-7-amido-4-methylcoumarin and 4-MUF-4-guanadinobenzoate, respectively (Vihinen and Mäntsälä 1989, Makoi and Ndakidemi 2008). 4-MUF-phosphate was used to assess phosphatase (Phos) activity.

Approximately 10 g of frozen glass beads were thawed on ice and subsequently transferred in a falcon tube containing 5 ml sterile water (MQ). Enzymes were released by vortexing the bead-water mix for 2 min. Three replicates of supernatant per resolved enzyme solutions were transferred into a 96 well microplate and substrate stock solution was added to get a final concentration of 400 µM (Findlay *et al.,* 2001). The remaining beads and MQ were dried at 60°C for 48 h to assess the exact weight of the beads. Fluorimetric enzyme assays were performed after adding the substrate over a time span of 24 h using a microplate reader (Tecan Infinite® 200, Switzerland). The excitation wavelength was 365 nm and fluorescence emission was measured at 445 nm. The microplates were stored on a plate shaker at 15°C between measurements. Fluorescence values were corrected for quenching by adding known quantities of MUF solution to the samples and comparing them to fluorescence increase when MUF was added to MQ of buffer (Findlay *et al.,* 2001). The reaction rates were calculated using the slope of the linear part of the fluorescence reaction curve. Potential enzyme activities were calculated with a standard curve and standardized to nmol substrate cm^{-2} bead area h^{-1}.

Data analysis

Community fingerprinting results (structure) and enzymatic activities (function) were analyzed using non-metric multidimensional scaling (NMDS). Bray-Curtis dissimilarities of Wisconsin square-root transformed community fingerprinting results and raw enzyme data were used for ordination. Vector fitting was applied to assess the gradients of enzymes in the community ordination and the operational taxonomic units (OTUs) specific to different communities. Factor fitting was used to depict differences between the two study sites and the influence of location, e.g. in-stream or riparian zone, on structure and function.

Clusters for structure and function were assessed for the different incubation periods. We chose the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as the clustering method due to its highest Pearson correlation between cophenetic distance (i.e. the intergroup distance from a dendrogram at which two observations are first combined into a single cluster) and the Bay-Curtis dissimilarity matrix. An optimal number of clusters was determined using the Rousseeuw quality index (Rousseeuw 1987). This index averages the silhouette width of a cluster, which is the average distance of an object to its cluster members compared to the distances to its next nearest cluster. The number of clusters with the highest average silhouette widths represents the best clustering. Additionally, mantel correlations between the original distance matrix and binary matrices from groups produced by cutting the dendrogram at different height levels were computed. The highest correlation equals the best clustering. Where the optimal number of clusters was not the same for both methods, silhouette widths were calculated for the range in the number of clusters and the largest number of clusters was chosen where no negative silhouette width (i.e. potential mismatch of objects in the clusters) occurred (Borcard *et al.,* 2011). The assessed clusters are depicted in the NMDS plots and geographic maps. To compare the goodness of the clustering results (i.e. approximately number of clusters) a Discriminant Analysis of Principal Components (DAPC) was performed after assessing the appropriate number of clusters (Jombart *et al.,* 2010). First the principal components (PC of a Principal Component Analysis (PCA)) of data undergo a successive K-means clustering with increasing numbers of clusters which are tested with the Bayesian information criterion. DAPC then constructs linear combinations of the PC of the original variables that have the largest between-group and the smallest within-group variance of the predefined clusters. A membership probability can be assessed based on the retained discriminant functions and indicate how tightly clusters are separated. The results of DAPC are presented in the supplementary material.

Permutational multivariate analysis of variance (PERMANOVA) was used to test the influence of the system (RC, SC), piezometer location (in-stream, riparian zone) and season on structure and function (Anderson 2001). Different models were built: A total model using all samples to test the effect of floodplain system and location, a model for the SC system testing influence of piezometer location and a model for the RC system to test the influence of piezometer location and season. For the RC system, additional models were built to assess differences between piezometer locations within each season and the effect of season for each location. A pairwise comparison between the two floodplain systems was also performed. Models were run on the same transformed data as used for the NMDS ordinations.

Directional spatial effects: Asymmetric Eigenvector Map (AEM) model

To understand to what extent directional processes as mediated by hydrologic water flow influenced bacterial community assembly (e.g. bacterial dispersal along the flow path) and ecosystem function (i.e. dependency of enzymatic activity on successive chemical processing of nutrients along the flow path), Asymmetric Eigenvector Map (AEM) models were produced for each sampling period (Blanchet *et al.,* 2008b, Blanchet *et al.,* 2011). Asymmetrical links between piezometers were summarized in a connectivity matrix and a weight on single links was introduced as a function of distance: $f(d_{ij}) = 1 - (d_{ij} / \max(d_{ij}))$, where d_{ij} represents the individual distances between piezometers (Dray *et al.,* 2006). Connectivity matrices were built based on assumed flow paths as assessed by relative water levels among piezometers.

The main direction of water flow was parallel to the stream at site RC and directed towards Lake Roseg at site SC (Appendix Figure 1). The connectivity matrices were therefore built by Delaunay triangulation (Delaunay 1934) with additional connecting links for within-stream piezometers (Appendix Figure 2). Spatial eigenfunctions were calculated by principal component analysis of the connectivity matrix (Blanchet *et al.,* 2008b). The eigenfunctions are then tested for positive spatial correlation based on Moran's I (Cliff and Ord 1981). The positively correlated eigenfunctions were then used as descriptors in the redundancy analysis (RDA) on structure and function as response variables. Forward selection of eigenvectors was performed to ensure a parsimonious model (Blanchet *et al.,* 2008a). Significance of constraints and the canonical axes were tested by the means of permutation tests

(999 permutations, "marginal" testing method) (Legendre *et al.,* 2011). Fitted values of the significant RDA axes were then plotted on the piezometer site maps using inversed distance weighted (idw) interpolation (Cressie 1993). Adjusted R^2 (R_a^2) was calculated to assess the explained variation by the models (Peres-Neto *et al.,* 2006).

Non-directional spatial effects: Moran's Eigenvector Map (MEM) *model*

Moran's Eigenvector Map (MEM) modeling was used to assess the influence of symmetric (spatially non-directed) processes influencing bacterial communities and functioning (Borcard and Legendre 2002, Borcard *et al.,* 2004, Dray *et al.,* 2006). MEM variables can cover mechanisms leading to spatial similarity patterns of structure or functions which are non-directional, i.e. the impact of non-directed dispersal of microbes or locally unique environmental structures on structure and function. We used the same connectivity matrix and weighting function as in the AEM models except for the lack of directionality. Principal coordinate analysis of the weighted Euclidian distance matrix derived spatial eigenfunctions (Borcard *et al.,* 2011). The eigenfunctions positively correlated in space (positive eigenvalues) were used in a subsequent RDA analysis following the procedure for the AEM. When both, forward-selected AEM and MEM eigenvectors, could significantly explain structure or function when used as explanatory variables, an additional variation partition was performed with partial RDA and the R^2 values were adjusted following Peres-Neto *et al.* (2006).

The response variables were Hellinger transformed prior to analysis and detrended for MEM modeling when necessary (Borcard and Legendre 2002, Borcard *et al.,* 2011). The R_a^2 values of the individual fractions are presented in the results. Spatial maps with the idw interpolations of MEM and AEM first RDA axis scores can be found in the supplementary material.

As we do not have specific environmental measures over the incubation period, we expect AEM and MEM variables to partly explain the impact of spatially structured environmental conditions within each floodplain matrix, such as soil water holding capacity, constant infiltration of groundwater or small-scaled physico-chemical variation as symmetrically-structured environmental factors. Larger scale physico-chemical gradients along the flow path would then be accounted as non-symmetrically structured environmental factors. We used forward selected enzyme activities as a surrogate explanatory factor of environmental constraints, e.g. nutrient availability, in variation partitioning together with AEM or MEM variables on bacteria assemblage structure. Two previous studies within the same systems revealed a high functional plasticity and readily induced change of enzymatic activities to altered environmental factors (Freimann *et al.,* under review-b, Freimann *et al.,* under review-a). The shared fraction of enzymes and spatial explanatory variables can give insight into the relative importance of structured "environmental" conditions on community assembly relative to, for example, directional and distance-proportional effects like e.g. bacterial dispersal. There is some degree of auto-correlation within this assumption, due to the potential association of bacterial assemblages with apparent enzyme activities. Regardless, potential auto-correlation should be minimized as enzymes were forward selected and therefore represent mostly non-redundant functional structures, thus any congruence of bacteria explained by spatial connectivity and non-redundant functions should indicate environmentally driven species sorting. In contrast, when non-redundant functions are not congruent with the spatial structure of bacterial communities, mass dispersal effects are more likely driving bacteria assemblage structure. The high plasticity potentially apparent in these habitats adds to the error robustness concerning auto-correlation. The non-explained variation by enzymatic activity on bacterial structure is additionally a measure of the community compositions functional potential.

Lastly, analysis of variance (ANOVA, Type III SS) and non-parametric Kruskal-Wallis analysis tested for differences in the number of OTUs and total enzyme activity, respectively, between floodplain systems and piezometer locations. All analyses were done using R statistical software (R Development Core Team, 2011).

Results

Structure and function at the Broad Spatial and Temporal Scale

Bacterial community structure differed between in-stream and riparian zone piezometer locations at site RC (PERMANOVA: $F_{1,77} = 6.61$, $P < 0.001$), whereas no difference between in-stream and riparian zone bacterial communities was found at site SC (PERMANOVA: $F_{1,15} = 1.19$, $P = 0.232$, Appendix Figure 3). There was no difference in bacteria communities between riparian-zone locations in SC and RC, but there were differences between RC riparian-zone locations and SC in-stream locations (PERMANOVA: $F_{1,69\text{-}71} = 1.13$ and 3.09, $P = 0.244$ and < 0.001, respectively). The OTUs fitted with highest significance on

the NMDS of enzymatic activities showed strong gradients towards the RC in-stream locations, pointing out the distinct bacterial assemblage at this location ($P < 0.001$, Appendix Figure 4).

The number of OTUs detected showed a dependence on the floodplain system and piezometer location (ANOVA: $F_{1,90} = 13.57$, $P < 0.001$) with a higher number of OTUs detected in RC in-stream locations compared to SC in-stream and RC riparian locations (Tukey's HSD: $P < 0.001$, Appendix Figure 3). The length of incubation had no influence on the abundance of detected OTUs, and there were no differences in OTUs between seasons at RC (ANOVA: $F_{2,72} = 0.31$, $P = 0.733$).

The total enzymatic activity showed differences between systems and locations with highest activities found in the in stream communities at RC (Kruskal-Wallis: $H=25.08$, $df=3$, $P<0.001$, multiple comparison: $P < 0.01$, Appendix Figure 3). The other system and locations did not differ in total enzyme activity (multiple comparisons: $P > 0.05$, appendix Figure 4). Most measured enzymes showed pronounced expression rates in the RC system as seen with the centroid locations relative to the enzyme scores in the function NMDS and the enzyme fitting on the structure NMDS ($P < 0.05$, Appendix Figures 3 and 4).

Enzymatic activities differed between RC and SC (PERMANOVAs: $F_{1,93} = 8.46$, $P < 0.001$, Appendix Figure 4). Enzyme activity patterns differed between the RC in-stream and riparian locations (PERMANOVA: $F_{1,77} = 11.60$, $P < 0.001$). At SC, there was no apparent difference in enzyme activity patterns between the in-stream and riparian zone locations (PERMANOVA: $F_{1,15} = 1.13$, $P = 0.326$).

Seasonal shifts in bacteria communities were apparent in RC in the riparian zone (PERMANOVA: $F_{2,62} = 1.51$, $P < 0.01$) but not in-stream locations (PERMANOVA: $F_{2,14,} = 0.71$, $P = 0.868$). The same pattern was also found for enzyme activity (PERMANOVA: $F_{2,62} = 4.67$, $P<0.001$ and $F_{2,14} = 1.03$, $P = 0.475$, respectively). Seasonal differences between piezometer locations was found for both structure and function at RC (PERMANOVAs: $F_{1,18\text{-}29} = 2.45$ to 9.36, $P < 0.05$, Figures 2, 3 and 4).

Spatial and temporal patterns of BCC and EF within the floodplains: Remo Creek

June

Community structure data revealed a separation of in-stream (IS) and riparian zone (RI) bacteria composition based on clustering in NMDS ordination and partially on UPGMA but not in the DAPC in June indicating little pronounced separation (Figure 3 A, Appendix Figure 9 A). IS sites in the mid-section of the stream were similar to RI bacteria composition. The few significantly fitted OTUs ($P < 0.01$ and $P < 0.001$) pointing towards the in-stream sites supports the finding of a subtle distinction of bacterial assemblage at this location (Figure 5 B). The AEM model explained 3.2% of the variation in BCC ($R_a^2 = 0.0322$, Pseudo-$F_{2,27} = 1.48$, $P = 0.012$, Appendix Figure 5 A). In contrast to the AEM models, the non-directional MEM models explained only 1.8% of bacteria assemblage structure ($R_a^2 = 0.0178$, Pseudo-$F_{1,28} = 1.53$,$P = 0.022$, Appendix Figure 5 B). Variation partitioning revealed 0.9% shared variation between the AEM and MEM models with no significant contribution of pure MEM variables (AEM: $R_a^2 = 0.0232$, $F_{2,27} = 1.34$, $P = 0.036$, MEM: $R_a^2 = 0.0087$, $F_{1,26} = 1.25$, $P = 0.145$, AEM ∩ MEM: $R_a^2 = 0.0090$, not testable).

In this June period, enzyme activity also showed a clear separation between locations and revealed an additional separation between riparian upstream and downstream piezometers (Figure 2 B, Appendix Figure 9 B). It seems that upstream riparian locations had higher enzymatic activities (Figure 2 B). Some 21.8% of the functional variation could be explained by directional spatial processes (AEM model: $R_a^2 = 0.2176$, Pseudo-$F_{3,26} = 3.69$: ,$P = 0.002$, Appendix Figure 5 C). The MEM model for enzyme activity was not significant.

When performing variation partitioning on BCC with BCC AEM and forward selected enzymes there was virtually no shared fraction apparent (AEM: $R_a^2 = 0.0319$, $F_{2,24} = 1.46$, $P = 0.02$, Enzymes: $R_a^2 = 0.072$, $F_{3,24} = 1.72$ $P = 0.005$, AEM ∩ Enzymes: $R_a^2 = 0.0003$, not testable).

July

Bacteria composition differed between in-stream and riparian zone locations in July in the NMDS ordination and the DAPC but not according to UPGMA clustering (Figure 3 A, Appendix Figure 10 A). However, enzyme activity was partly separated between locations in the NMDS, DAPC and the UPGMA clustering (Figure 3 A, Appendix Figure 10 B). No significant AEM models could be

built for structure or function in July. The MEM model for bacteria structure explained a small portion (2.6%) of the total variation (R_a^2 = 0.0256, Pseudo-$F_{1,17}$ = 1.47, P = 0.047, Appendix figure 6) and there was no significant MEM model for enzyme activity in July.

October

In October, there seemed to be increasing bacteria Beta-diversity in the floodplain as seen by the wider dispersion in the NMDS, the larger number of formed UPGMA clusters and the high membership probability of the 7 fitted clusters in DAPC (Figure 4 A, Appendix Figure 12 A). Still apparent was the difference between in-stream and riparian locations in bacteria composition. No significant AEM model could be built for bacteria structure. The MEM model explained 1.3% of the bacteria structure (R_a^2 = 0.0129, Pseudo-$F_{1,27}$ = 1.37, P = 0.034, Appendix Figure 7 A). Enzyme activity was different between in-stream and riparian locations but showed some overlap in the NMDS, DAPC and UPGMA clusters (Figure 4 B, Appendix Figure 10 B). The AEM model explained 9.3% of the variation in enzyme activity (R_a^2 = 0.0932, Pseudo-$F_{1,27}$ = 2.44, P = 0.028, Appendix Figure 7 B), whereas the MEM model explained 5.3% (R_a^2 = 0.0534, Pseudo-$F_{1,27}$ = 2.57, P = 0.042, Appendix Figure 7 C). Variation partitioning showed that the MEM model shared all the explained variation with the AEM model, but the fraction explained only by the AEM model was not significant (AEM: R_a^2 = 0.0238, $F_{2,27}$ = 1.35, P = 0.227, MEM: R_a^2 = - 0.0160, $F_{1,25}$ = 0.55, P = 0.711, AEM ∩ MEM: R_a^2 = 0.0694, not testable).

There was no symmetrically spatial-structured enzyme activity (shared variation) explaining bacteria assembly (MEM: R_a^2 = 0.0198, $F_{1,26}$ = 1.57, P = 0.013, Enzymes: R_a^2 = 0.0414, $F_{3,24}$ = 2.18 P = 0.005, MEM ∩ Enzymes: R_a^2 = - 0.0069, not testable)

Spatial and temporal patterns of BCC and EF within the floodplains: Simone Creek

Although PERMANOVA did not show a significant difference between in-stream and riparian zone locations in bacteria assemblage structure at SC, there was a trend for differentiation (see dispersions ellipses in Figure 5 A). There was a subtle change in bacteria composition towards Lake Roseg as seen with the annotated UPGMA clusters and the DAPC (Figure 5 A, Appendix Figure 12 A). This pattern could be modeled with the AEM, which explained 3.5% of the explained variation in bacteria composition (R_a^2 = 0.0335, Pseudo-$F_{1,14}$ = 1.52, P = 0.027, Appendix figure 8 A).

Enzyme activity showed the same patterns as for bacteria structure: no significant separation between in-stream and riparian locations but a gradient of annotated UPGMA cluster separation towards Lake Roseg (see successive change of the clusters along the NMDS axes and the first discriminant function in Figure 5 B and Appendix Figure 12 B, respectively). The directional AEM model explained 40% of the variation in enzyme activity (R_a^2 = 0.3996, Pseudo-$F_{2,13}$ = 5.99, P=0.002, Appendix figure 8 B). No significant MEM models could be built for bacteria structure or function for Simone Creek.

The variation partitioning with forward selected enzymes and BCC AEM model revealed a partly congruent structured fraction of enzymatic activity to the BCC (AEM: R_a^2 = 0.0063, $F_{1,12}$ = 1.09, P = 0.32, Enzymes: R_a^2 = 0.0534, $F_{2,12}$ = 1.41 P = 0.017, AEM ∩ Enzymes: R_a^2 = 0.0272, not testable).

Discussion

We found high similarity in bacteria assemblage structure in riparian zone locations of both floodplains but differences between in-stream bacteria structure between the two floodplains. This result suggests the importance of site characteristics in influencing bacteria composition and occurrence. For instance, there may have been less variation between floodplain bacteria community in the riparian zone due to similar controlling factors such as soil pH and homogenous soil grain-size distribution (Fierer and Jackson 2006, Freimann *et al.,* under review-b).

The degree of separation between in-stream bacteria structure in the two floodplains reflects the importance of hydrologic connectivity within each floodplain. The SC floodplain showed a significant directional change in bacteria assemblage towards Lake Roseg that was independent of the piezometer locations (see also appendix Figure 8 A). This pattern could be due to a pronounced water flow through the floodplain perpendicular to the lake (see appendix Figure 1). Stream meandering within the SC floodplain may have allowed more exchange with the riparian zone. I.e. groundwater flow is directed across the floodplain streams and multiple small temporal tributaries, thus more exchange of in-stream groundwater, in-stream water and riparian water can be expected. This is different to the RC floodplain, where the direction of the channel and the groundwater flow coincided over a large part of the study

area. This allows for a separation of sediment groundwater flow which is in exchange with the channel water at SC versus a riparian water flow that is fed by lateral inflow with less influence from the channel at RC. Previously studies have shown that bacteria can be washed from soils and be transported into streams (Cousin 2009). Such hydrologically driven inputs can even influence larger ecosystems such as lakes, thus potentially drive the lake community toward resembling the in-stream community (i.e. mass effect) (Lindström and Bergström 2004, Crump *et al.,* 2007). The effects of multiple opportunities for in- and exfiltration into the riparian zone across the floodplain could explain the less pronounced separation of in-stream vs. riparian locations in bacteria structure at SC. This situation is less apparent at RC floodplain with its relatively straight channel and clearly separated bacterial community structures.

A striking result was that bacteria function showed the same pattern as bacteria structure at SC with the AEM model explaining a large part of the enzymatic activity. (Appendix Figure 8 B). The successive transformation of, e.g., dissolved organic matter along the flow path may drive the variation in bacteria function (Sobek *et al.,* 2003, Ayuso *et al.,* 2011). Locations situated closer to the lake showed pronounced activity in Est, *Bet*, *Nac* and *Epep*, whereas more distant sites showed high enzymatic activities of *Alph*, *Xyl* and *Leu* (Figure 5 B). The fact that the distant sites had less pronounced enzymatic activity for some particular enzymes could be due to the primary use of easily degradable molecules at these locations, whereas bacteria at locations near the lake may need to invest more into enzyme activity to access the remaining and less accessible nutrients provided by upstream waters. Those enzymes degrading more complex compounds were increasingly found at the downstream end of the floodplain fit this hypothesis. For example, *Bet* has been shown to be regulated by the quantity and quality of polymeric substances and be induced by recalcitrant glycoside containing substrates (Miettinen *et al.,* 1996, Misic *et al.,* 2002). Miettinen *et al*. (1996) also found that collapsing phytoplankton blooms that release polysaccharides stimulate *Bet* activity. In late autumn, there could be infiltration of such polysaccharides from Lake Roseg into the riparian zone, further affecting *Bet* activity at locations near the lake. Also, hemicellulose degrading enzymes such as *Xyl* can be more pronounced at organic matter rich upwelling zones (Rulík and Spáčil 2004).

The directional change in bacteria function was more pronounced than the directional change in bacteria community assembly as the AEM models explained a higher amount of the variation in function. This result implies functional plasticity to be apparent within these microbial communities. It has previously been shown that groundwater driven systems in alpine floodplains are inhabited by bacteria having a generalists strategy with distinct functional plasticity (Freimann *et al.,* under review-b). Furthermore, a stochastic community assembly with disconnected functionality was examined in an early successional stream by Frossard *et al.* (2012). They argued that the stochastic distribution of bacteria could be caused by a high dispersal rate and different dispersal and colonization abilities, which in turn can override the influence of local environmental differences and ultimately diminish spatial structural patterns in bacteria assembly. Although the bacterial assemblage seems to be partly driven by water flow, thus incorporating potential dispersal (i.e. significant AEM model for BCC), there is no indication of the process leading to a stochastically assembled bacteria community at SC. As environmental constraining factors seem to be dependent on spatially directed change in availability of, e.g., nutrients or organic matter that are mostly related to water flow in this system, they were incorporated in the functional AEM model. Thus, bacteria assemblage structure may be driven by species sorting mechanisms as they changed in congruence with bacteria function.

Mass dispersal effects could also contribute to bacteria assemblage structure, but would mainly depend on species-specific strategies. As generalists seem to dominate the SC floodplain, one should see covariation of Beta diversity along the dispersal route as a consequence of mass effects, whereas specialists still would be more affected by the environmental gradient (see figure 1 in Lindström and Langenheder 2011) (Figure 5 A, composition change along NMDS axis-1). As specialist species would show covariation in beta diversity along an environmental gradient (which is, in turn, highly linked to the hydrological flow path in this study), it is difficult to make predictions which mechanism contributes to what extend to community assembly. Nonetheless, when variance partitioning was performed with enzyme activities and selected AEM variables for the model for bacteria community structure, the forward selected (potentially “non-redundant”) enzymes with asymmetric spatial structure (shared fraction) contributed around four times more to the explained variation in community assembly than the BCC AEM variables alone. This may indicate the relative importance of directionally-structured environmental constraints on potential bacteria specialists driving beta diversity.

Enzyme activity showed a pronounced longitudinal change along the riparian zone with lower total enzymatic activity downstream (Figure 2 B, Appendix Figure 9 B). This longitudinal change in enzymatic activity may be driven by the same mechanisms as at SC floodplain, i.e. a successive shift in nutrient and organic matter resources to a more recalcitrant form along the flow path. For instance, *Epep* and *Est* activity also showed more activity at locations further down-stream compared to enzymes that degrade more bioavailable compounds at up-stream locations. *Nac* was more active for in-stream bacteria assemblages, although

not being observed at SC. The occurrence of, e.g., diatoms may drive this enzyme at in-stream locations at RC, whereas lake water infiltration may transport N-acteylglucosamines of algal origin into the SC floodplain, thus separating sites near the lake in respect to function (Durkin *et al.,* 2009). AEM explained up to 21% of the variation in bacteria function, thus a longitudinally structured environmental gradient is likely driving enzymatic activity (Appendix Figure 5 C). When variation partitioning with enzyme activities and AEM variables are performed on bacteria community assembly, such as for the SC floodplain above, there is virtually no variation explained in community assembly by the expression of enzymes that show similar asymmetrical spatial structures as the bacterial community itself, i.e. the shared fraction of explained variance (as a surrogate of environmental condition). This finding and the fact that the variation partitioning on bacterial community revealed no significant influence of pure symmetric MEM explanatory variables but significantly contributing asymmetric AEM variables, supports the idea of bacterial community assembly mainly driven by a water flow directed mechanism and smaller impact of flow mediated environmental constraints as apparent within the SC floodplain. Regardless, the finding that enzymes are not structured the same way as bacteria assemblage structure (see also appendix Figure 5 A and 5 C) suggests a high degree of functional plasticity and thus a dominance of generalists. This fact along with a relatively small covariation in beta diversity along the flow path (structured change along NMDS axis-1 in Figure 2 A), suggests a flow driven patch dynamic over species sorting as an underlying community assembly mechanism.

As the snowmelt ablation period during spring gives way to a more arid period during summer, water saturation within the RC floodplain decreases. This drying pattern is reflected in reduced water potentials measured in the piezometers. The AEM models were not significant for the summer period, likely due to a lack of hydrological linkages that drive bacteria community assembly and function. Furthermore, in-stream bacteria structure was distinguished from riparian zone bacteria structure in the NMDS and DAPC approach, although not being clearly separated with the UPGMA cluster analysis (Figure 3 A, Appendix Figure 10 A). More subtle, but captured by the MEM model (explaining 2.6% of the variation in bacteria structure), there also seems to be a distinction between left and right side riparian zones in RC (Appendix Figure 6). This undirected spatial mechanism may represent local environmental factors that differ between the two riparian zones. For instance, the presence of the adjacent coniferous forest on the left side of the stream may influence local environmental conditions such as modifying temperatures during the day. Regardless, the use of enzymes as a surrogate of environmental explanatory factors in an RDA for community assembly was not significant (Pseudo-$F_{8,10}$ = 0.97, P = 0.58), thus weakening the idea of strong environmental constraints influencing bacterial communities but supporting the idea of non-directed small-scale dispersal (e.g. during occasional precipitation events) driving local differences via patch dynamic or neutral mechanisms and generally high functional potential.

Bacteria function was generally different between the in-stream and riparian zone locations with higher enzyme activities in the in-stream locations (Figure 3 B, Appendix Figure 10 B)). The two in-stream locations that showed the stronger interaction with the riparian zone during spring (RCP18 and RCP21) were also more functionally similar to riparian locations in summer. Apart from this difference, there was no directional gradient in bacteria function as apparent during the hydrologically active snowmelt ablation period.

Community assembly mechanisms during this period of low hydrologic activity in summer could be driven by weak species sorting induced by subtle environmental differences but also via small-scale dispersal of bacteria. These mechanisms can potentially be captured but not distinguished with the MEM model. The fact that bacterial communities were not strongly distinguished and performed equal functioning is likely at least partially a historical consequence of the apparent dispersal of bacteria communities throughout the riparian zone during the ablation period.

As precipitation increases towards autumn, the riparian zone at RC becomes rewetted or saturated. Community diversity in the riparian zone is highly pronounced but did not show a directional pattern along the emerging flow path, thus no significant AEM model could be built. The MEM model revealed a pattern, which again seemed to separate the left and right riparian zones (Appendix Figure 7 A) but with minimal explanatory power (1.3%). In contrast to bacteria structure, bacteria function showed directional spatial patterns that are driven by both, directional and non-directional structure (9% and 5% of the explained variance in function, respectively, appendix Figure 7 B and C). The variation partitioning revealed no independent explanatory power of MEM variables, indicating mainly spatially-directed structure in bacteria function.

There was no congruent symmetrically spatial-structured enzyme activity with the MEM explaining bacteria assembly thus suggesting minimal spatially-structured environmental constraints affecting bacteria composition and a high degree of functional

plasticity. Lastly, the variation of the MEM model explaining bacteria structure in autumn decreased by around 50% compared to the summer season. This suggests a reduced importance of locally structured environmental factors and/or symmetrical dispersal.

Apparent local and stochastic diversification of bacteria assemblages may be a historical consequence remaining from the low hydrologic connectivity period. The presence of generalists in the floodplain that shows few environmental differences would favor an increasing diversity during low hydrologic connectivity periods leading to a stochastic pattern in community assembly (Lindström and Langenheder 2011).

As bacteria function reacts quickly to changing nutritional states, this may be a cue for an initiating the restructuring of asymmetric environmental conditions driven by hydrology. Although the AEM model could not explain bacteria community structure at the sampled time point, it is likely that emerging regional effects will become important as hydrologic linkages persist for a longer period. At the sampling time point, there may be priority effects interfering with forthcoming regional effects (Loeuille and Leibold 2008, Urban and De Meester 2009). I.e. the monopolization of resources or local adaptive evolution can reduce the establishment success of invading and competing species. Another possible explanation for the increased diversity is that the emerging hydrological connectivity may favor bacterial cells that have been dormant during the low hydrological period and become active with rewetting of the floodplain (Lennon and Jones 2011).

Concluding remarks

This study provides insight into the importance of hydrology driving bacterial community assembly and associated function within alpine floodplains. The interplay between general landscape differences with seasonally changing water dynamics strongly influences bacteria community assembly and function, thus affecting ecosystem services in space and time. Disentangling local and regional effects as underlying mechanisms and their relative contribution to community assembly is one of the great challenges in microbial community ecology. Measuring bacteria dispersal in natural habitats is difficult. Floodplains potentially represent excellent systems to examine the importance of passive bacteria dispersal. However, the co-variation of environmental factors along a dispersal route within the water column makes it difficult to clearly define the importance of dispersal and environmental factors as mechanisms for assembly of bacterial communities and related functioning. There are relatively few empirical field and experimental studies examining the effect of dispersal on bacteria community assembly and function (see i.e. Lindström and Östman 2011 and references herein).

The empirical approach used in this study towards understanding dispersal and species sorting mechanisms is somewhat speculative due to potentially high degree of co-variation in spatial models, not measured and potentially during the incubation period variable physico-chemical parameters and difficulty in assigning true bacterial dispersal. Regardless, the temporal and structural aspects included in this study give some perspective of how the interplay of different mechanisms can influence bacteria assemblages in alpine floodplains. Future studies should include temporal and spatial changes in essential chemical compounds such as types of dissolved organic matter to assess the impact of successional changes within flow paths on biota. Also, soil properties would be helpful to better understand environmental structuring on community assembly, which also can co-vary in the spatial models. Lastly, an assessment of the exact hydrology (i.e. connectivity and flow velocity) could improve greatly the quality of the used spatial models.

Ecosystem functions and biodiversity can be tightly coupled and both may get altered when i.e. hydrology changes within alpine floodplains. Anyway our results showed that spatial mechanisms only explained a small part of the variance in BCC whereas EF was greatly influenced by hydrological mediated mechanism. We believe that a better understanding of bacterial community assembly, dynamics and function within floodplains or meadows could contribute towards the development of optimal strategies in floodplain and wetland management to conserve or even improve specific ecosystem functions.

Acknowledgements

We thank Tobias Vogt for helping with the geo-positioning and assessing the hydrological conditions, and Simone Blaser and Christa Jolidon for field assistance. This study was funded by the Swiss National Foundation (No. 31003A-119735).

Titles and legends to figures

Figure 1

Maps of the Val Roseg catchment and locations of the study floodplains. Locations of the piezometers in each floodplain relative to each stream and the direction of flow paths are depicted.

Figures 2 to 5

NMDS of ARISA profiles, enzymatic activities and associated UPGMA clusters for the specific floodplains and different seasons. **Figures 2 to 4:** Site RC in June, July and October, respectively. **Figure 5:** Site SC in October. **Panels A:** NMDS and map of ARISA profiles. **Panels B:** NMDS and map of enzyme activities. The size of symbols is relative to the number of OTUs or the square-root of the sum of the logarithmic enzyme activities at the specific piezometer location, respectively. Colors of symbols correspond with the assigned UPGMA cluster for ARISA or enzymatic activity patterns. Dispersion-ellipses depict the standard error of weighted average scores of piezometer location (IS = in-stream, RI = riparian zone, confidence limit = 0.95). OTUs are fitted as arrows with a fitting power of $P < 0.01$ (grey arrows) and $P < 0.001$ (blue arrows). Enzyme activities are fitted as arrows with fitting power $P < 0.05$ depicted in blue. Geographical locations of piezometers are depicted on the right side. Symbols correspond to the UPGMA cluster assignment or ARISA and enzymatic activity patterns, respectively.

Figure 1

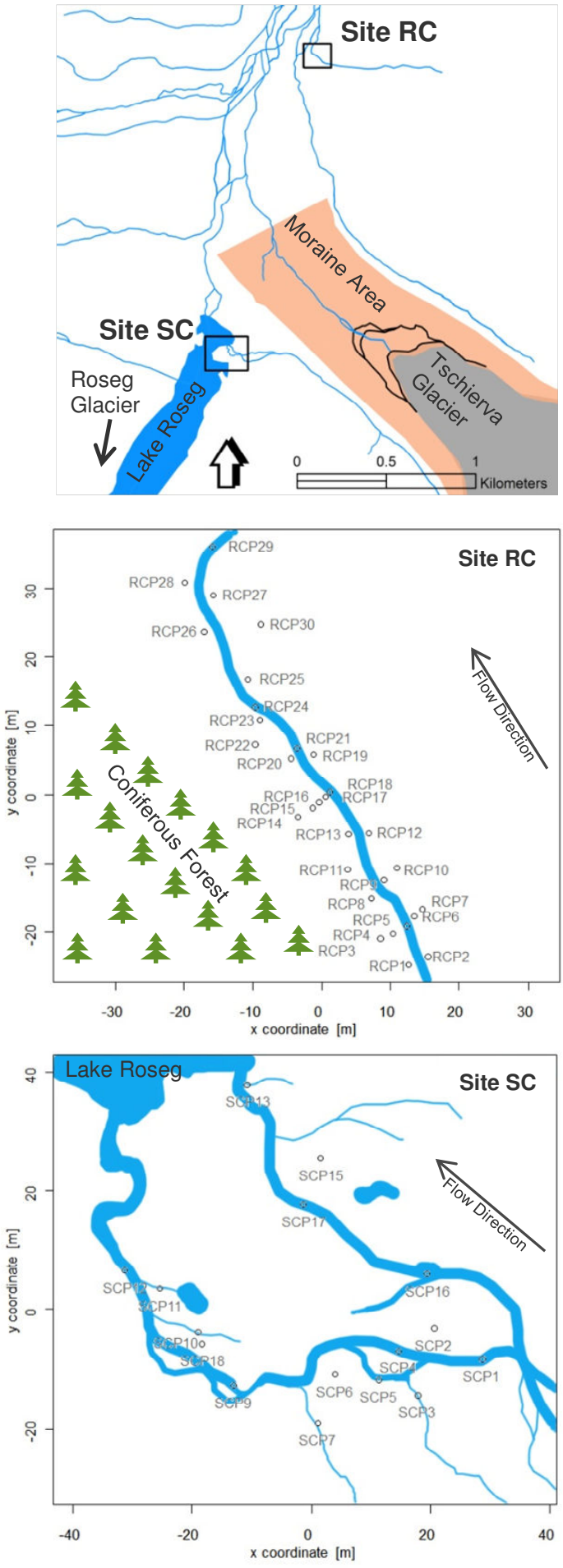

Site RC
Moraine Area
Site SC
Roseg Glacier
Lake Roseg
Tschierva Glacier
0
0.5
1
Kilometers
Site RC
RCP29
RCP28
RCP27
RCP26
RCP30
RCP25
RCP24
RCP23
RCP22
RCP21
RCP20
RCP19
RCP18
RCP16
RCP17
RCP15
RCP14
RCP13
RCP12
RCP11
RCP10
RCP9
RCP8
RCP7
RCP6
RCP5
RCP4
RCP3
RCP1
RCP2
Flow Direction
Coniferous Forest
y coordinate [m]
x coordinate [m]
Lake Roseg
Site SC
SCP13
SCP15
SCP17
SCP16
SCP12
SCP11
SCP10
SCP18
SCP9
SCP6
SCP5
SCP4
SCP2
SCP1
SCP3
SCP7
Flow Direction
y coordinate [m]
x coordinate [m]

Figure 2

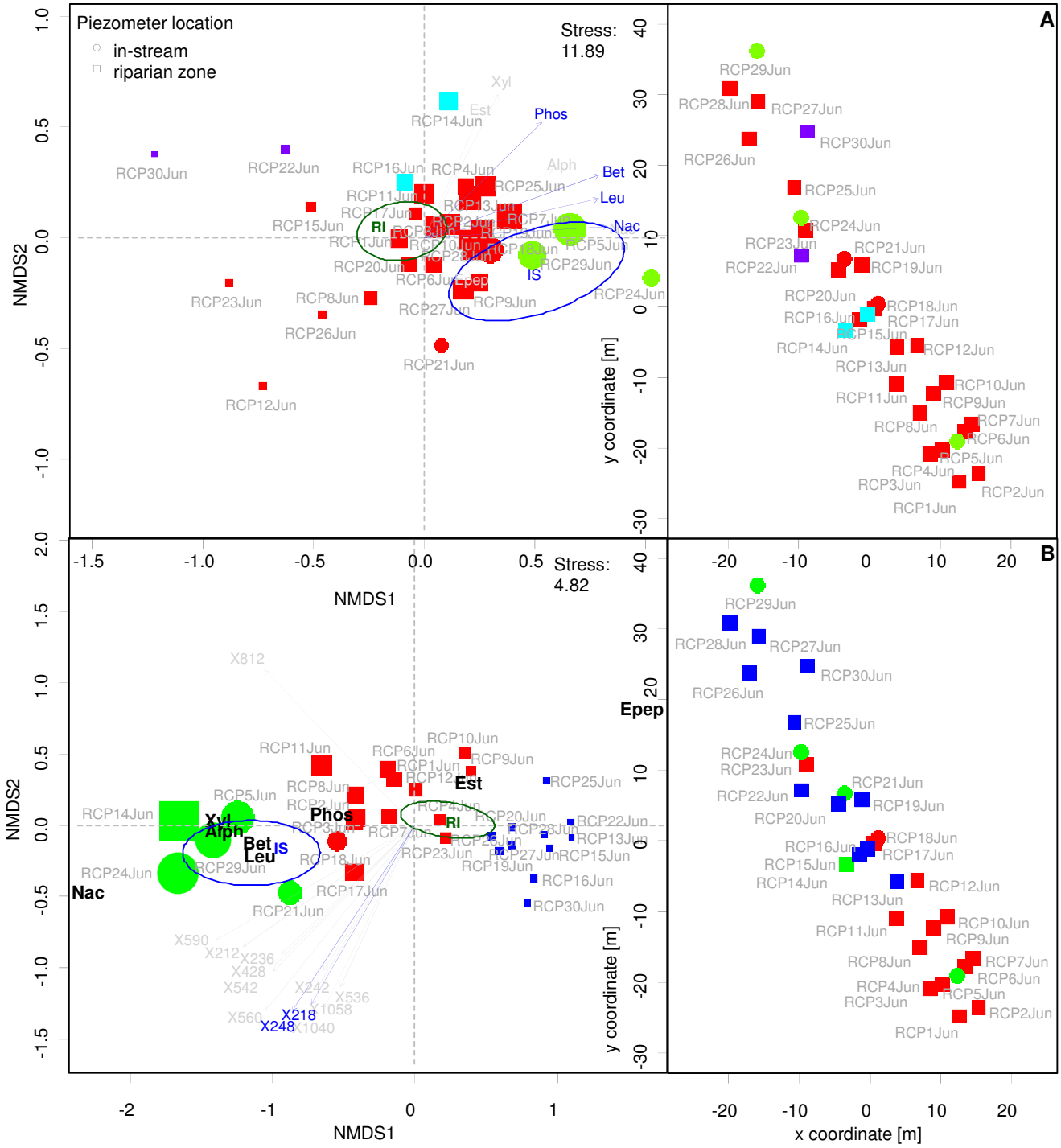
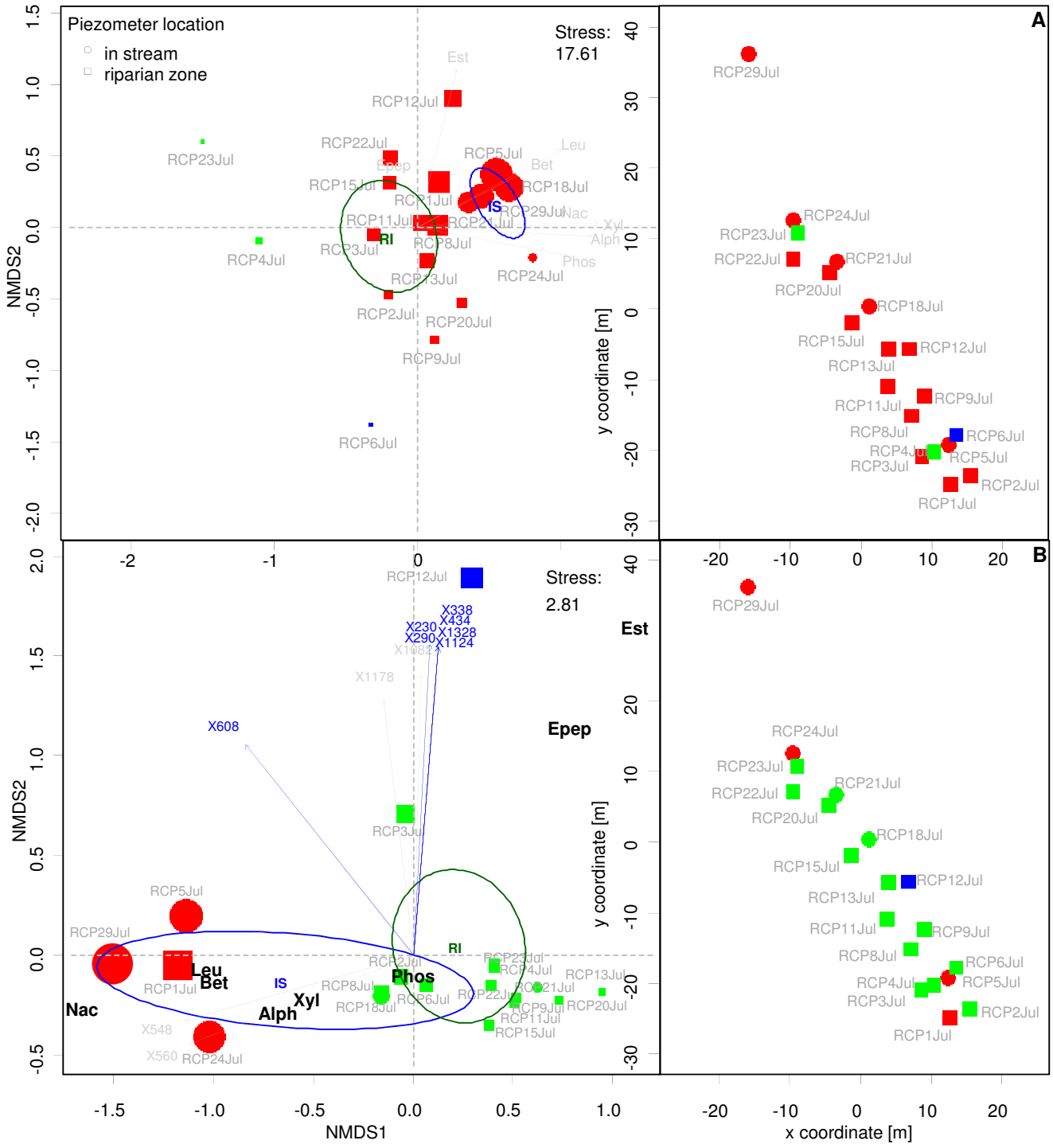

Piezometer location
in-stream
riparian zone
Stress:
11.89
A
Xyl
Est
Phos
Alph
Bet
Leu
Nac
RI
IS
Epep
NMDS2
y coordinate [m]
Stress:
4.82
B
NMDS1
x coordinate [m]

Figure 3


Piezometer location
in stream
riparian zone
Stress:
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A
RCP29Jul
RCP24Jul
RCP23Jul
RCP22Jul
RCP21Jul
RCP20Jul
RCP18Jul
RCP15Jul
RCP12Jul
RCP13Jul
RCP11Jul
RCP9Jul
RCP8Jul
RCP6Jul
RCP4Jul
RCP5Jul
RCP3Jul
RCP2Jul
RCP1Jul
Est
Leu
Epep
Bet
Nac
Xyl
Alph
Phos
IS
RI
y coordinate [m]
NMDS2
Stress:
2.81
B
X338
X434
X230
X1328
X290
X1124
X1178
X608
X548
X560
x coordinate [m]
NMDS1

Figure 4

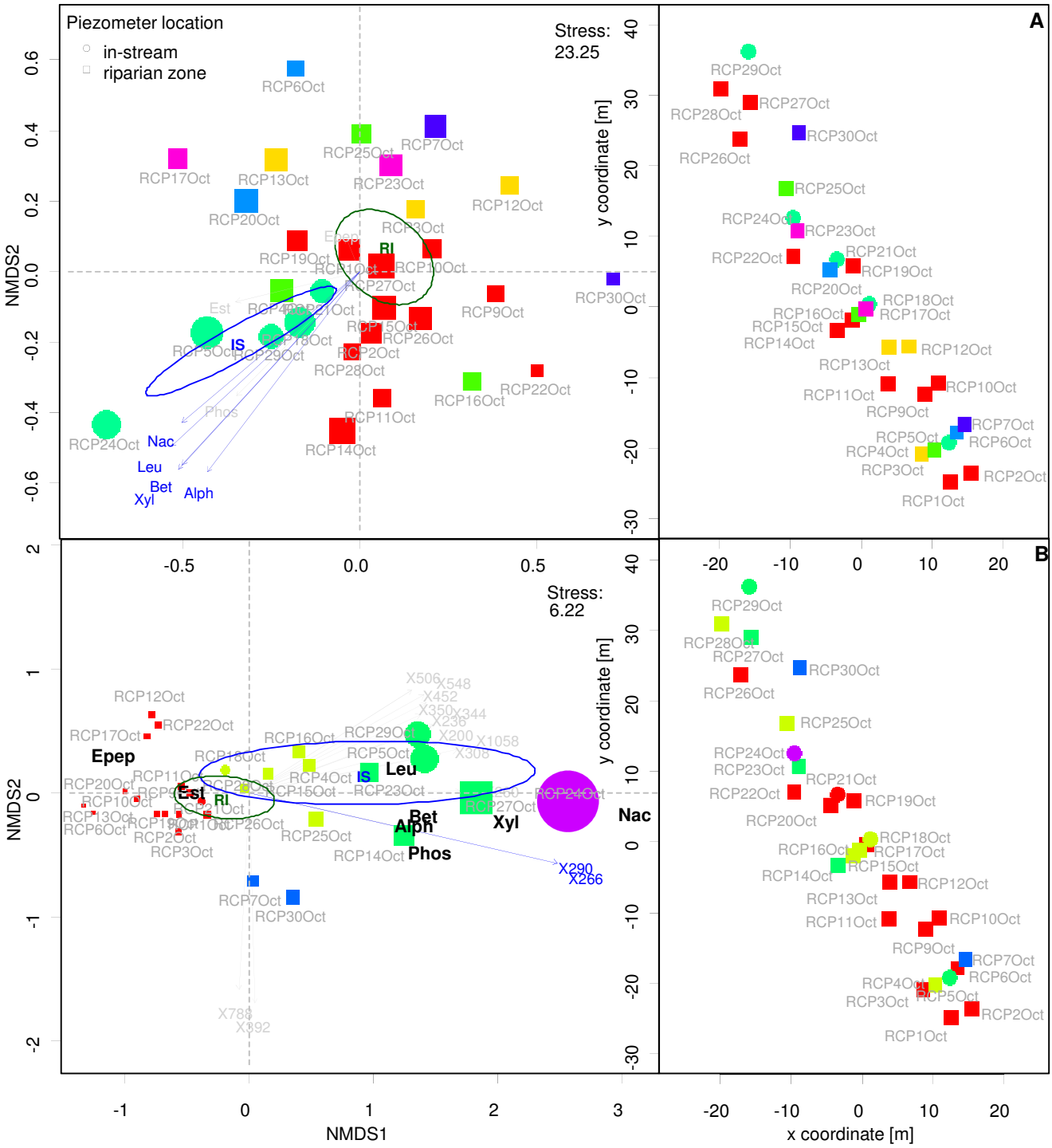
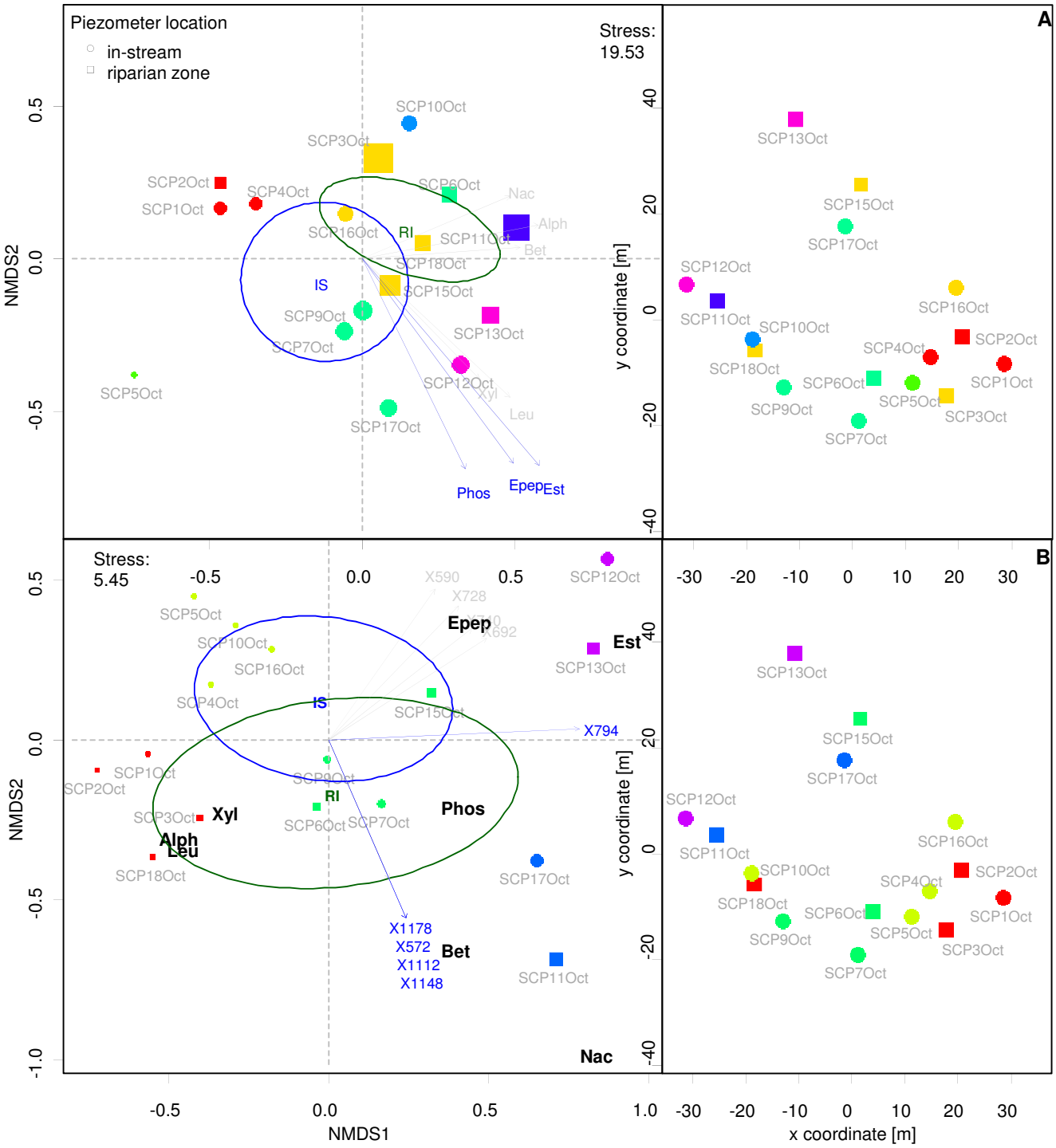

Piezometer location
in-stream
riparian zone
Stress:
23.25
A
Stress:
6.22
B
NMDS1
NMDS2
x coordinate [m]
y coordinate [m]
Epep
Est
RI
IS
Nac
Leu
Bet
Xyl
Alph
Phos

Figure 5


Piezometer location
in-stream
riparian zone
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A
SCP10Oct
SCP3Oct
SCP2Oct
SCP4Oct
SCP1Oct
SCP6Oct
Nac
Alph
Bet
SCP16Oct
RI
SCP11Oct
SCP18Oct
IS
SCP15Oct
SCP9Oct
SCP13Oct
SCP7Oct
SCP5Oct
SCP12Oct
Xyl
Leu
SCP17Oct
Phos
Epep
Est
NMDS2
y coordinate [m]
Stress:
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B
X590
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X692
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X572
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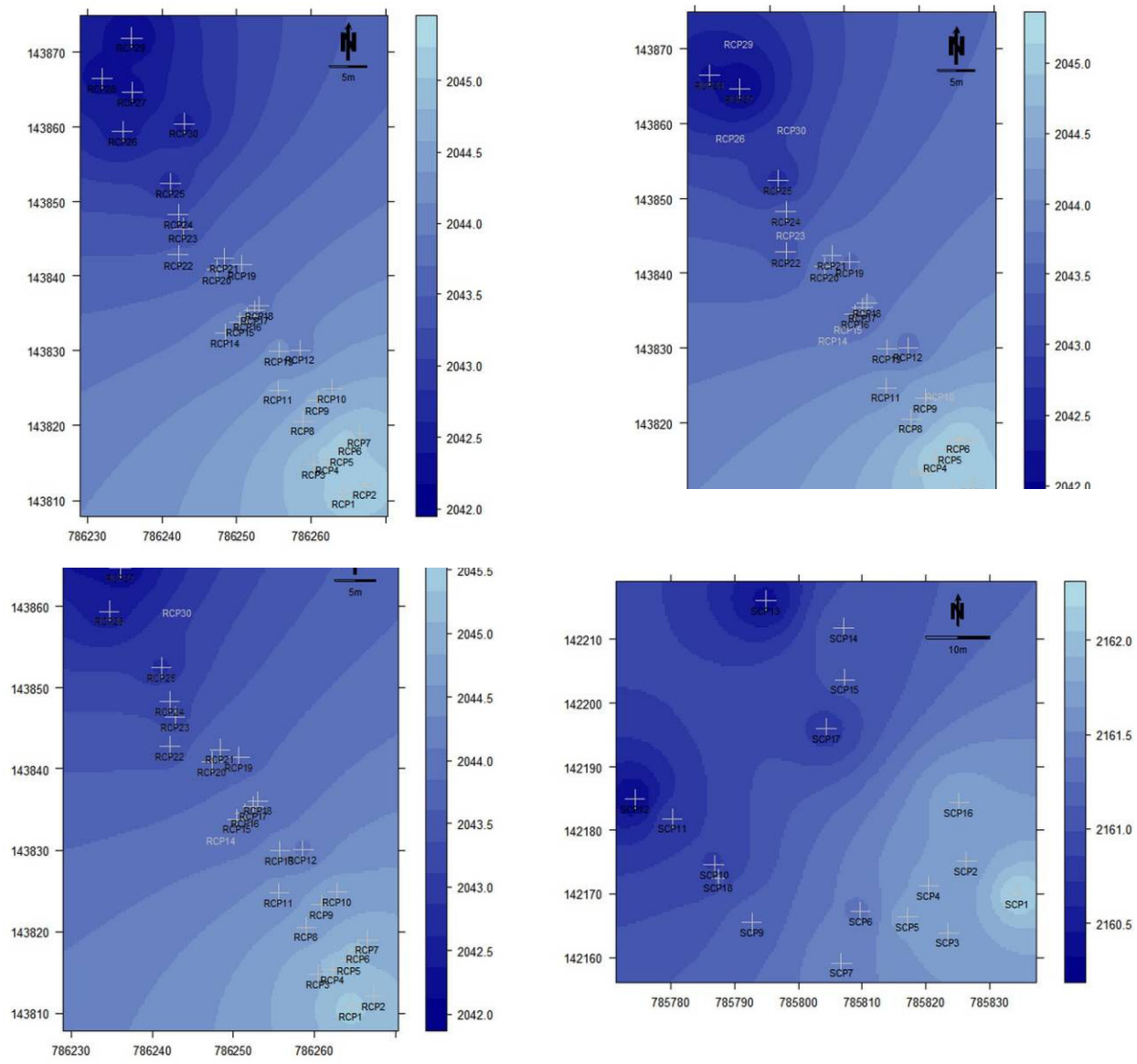
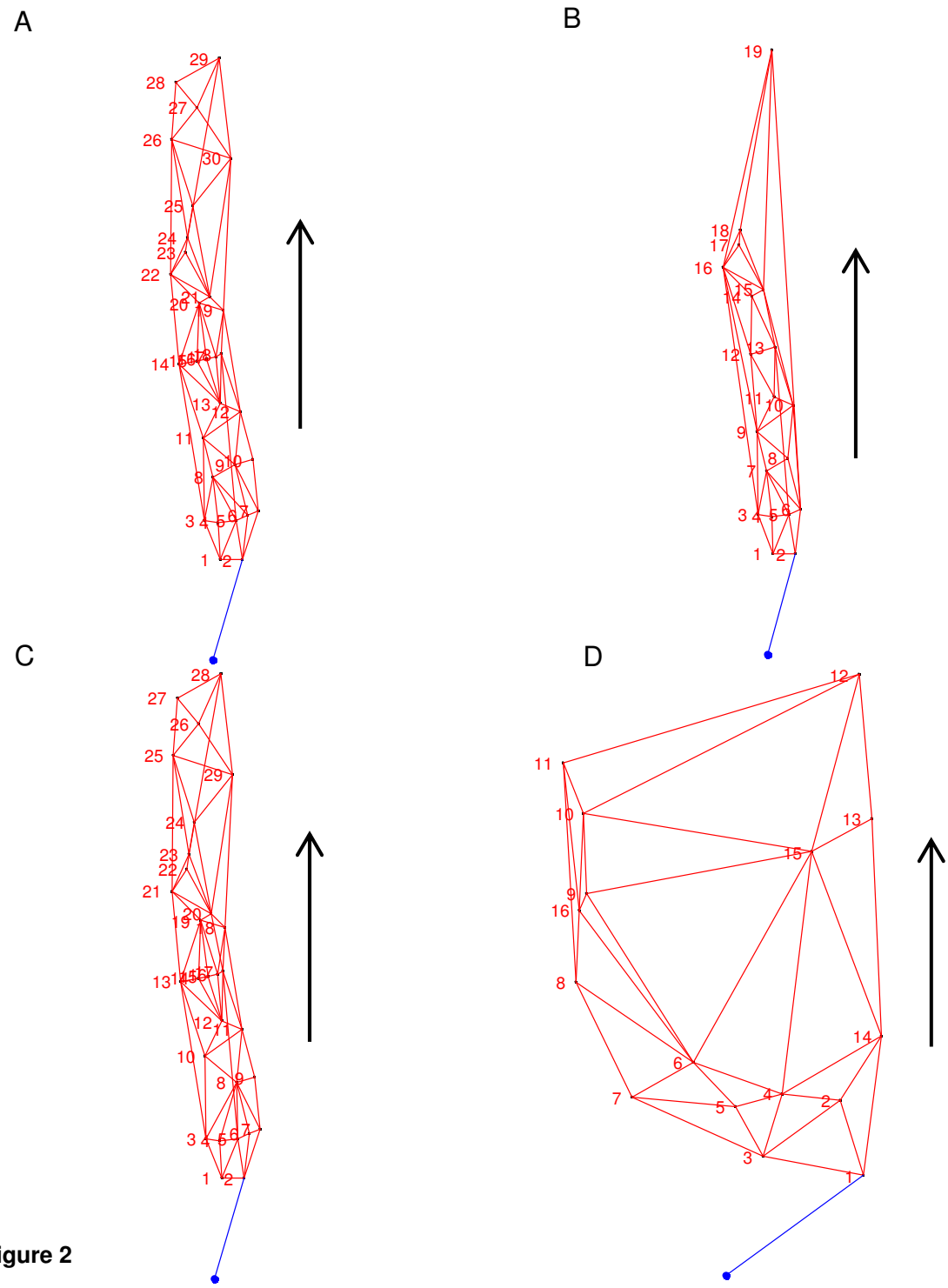
NMDS1
x coordinate [m]

Supplementary information



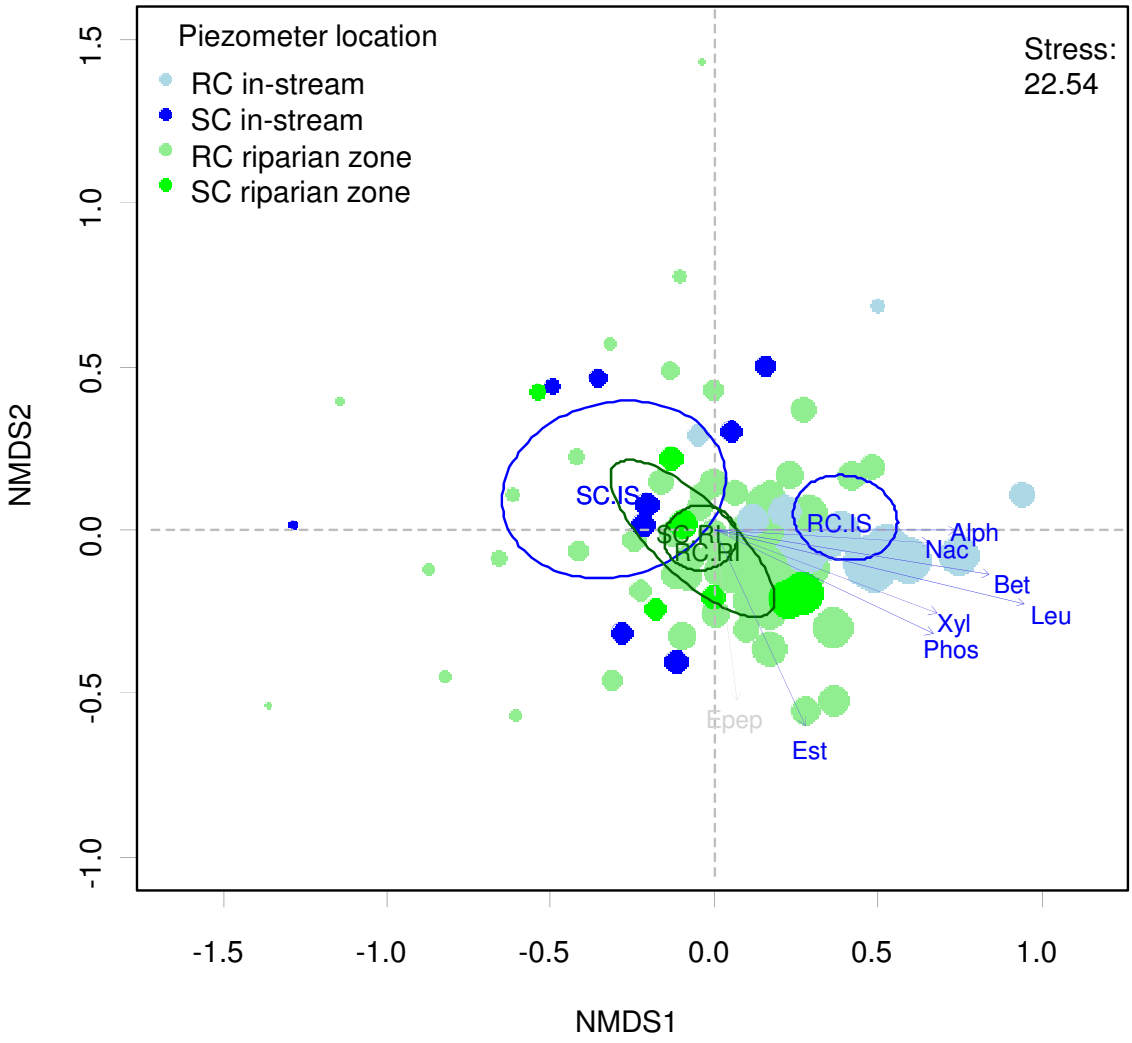
Appendix Figure 1

Measured water potentials within the piezometer during the sampling periods and their interpolated surface as assessed by inverse distance weighting interpolation for site RC in June (A), July (B) and October (C) and site SC (D). Scale bar on the right site depicts elevation above sea level and axes are fitted to the Swiss Grid coordinate system.



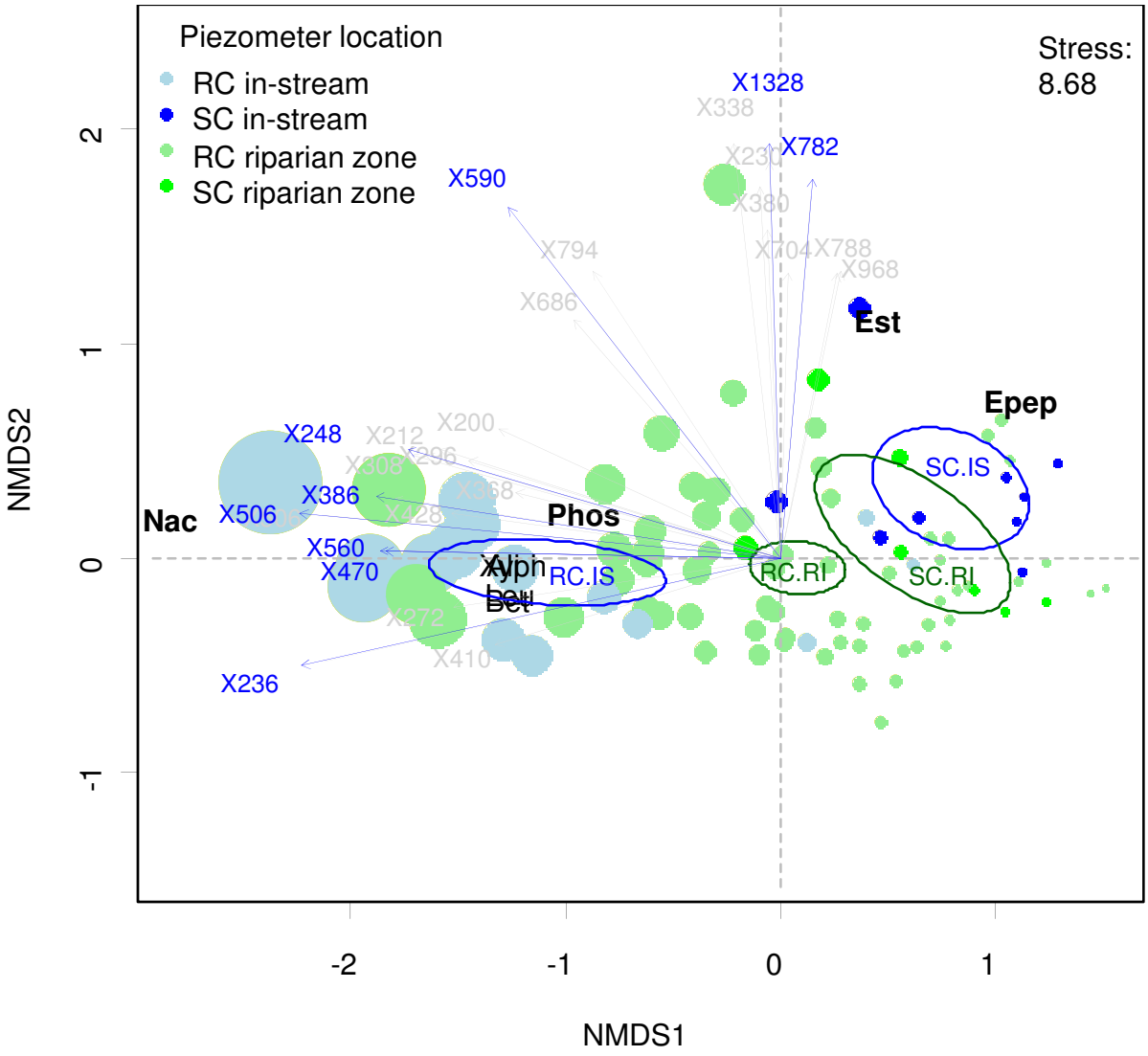
Appendix Figure 2

Connectivity matrices used for AEM and MEM models at site RC during June (A), July (B) and October (C) and at site SC during October (D). The arrows indicate the directions used for asymmetric spatial models.



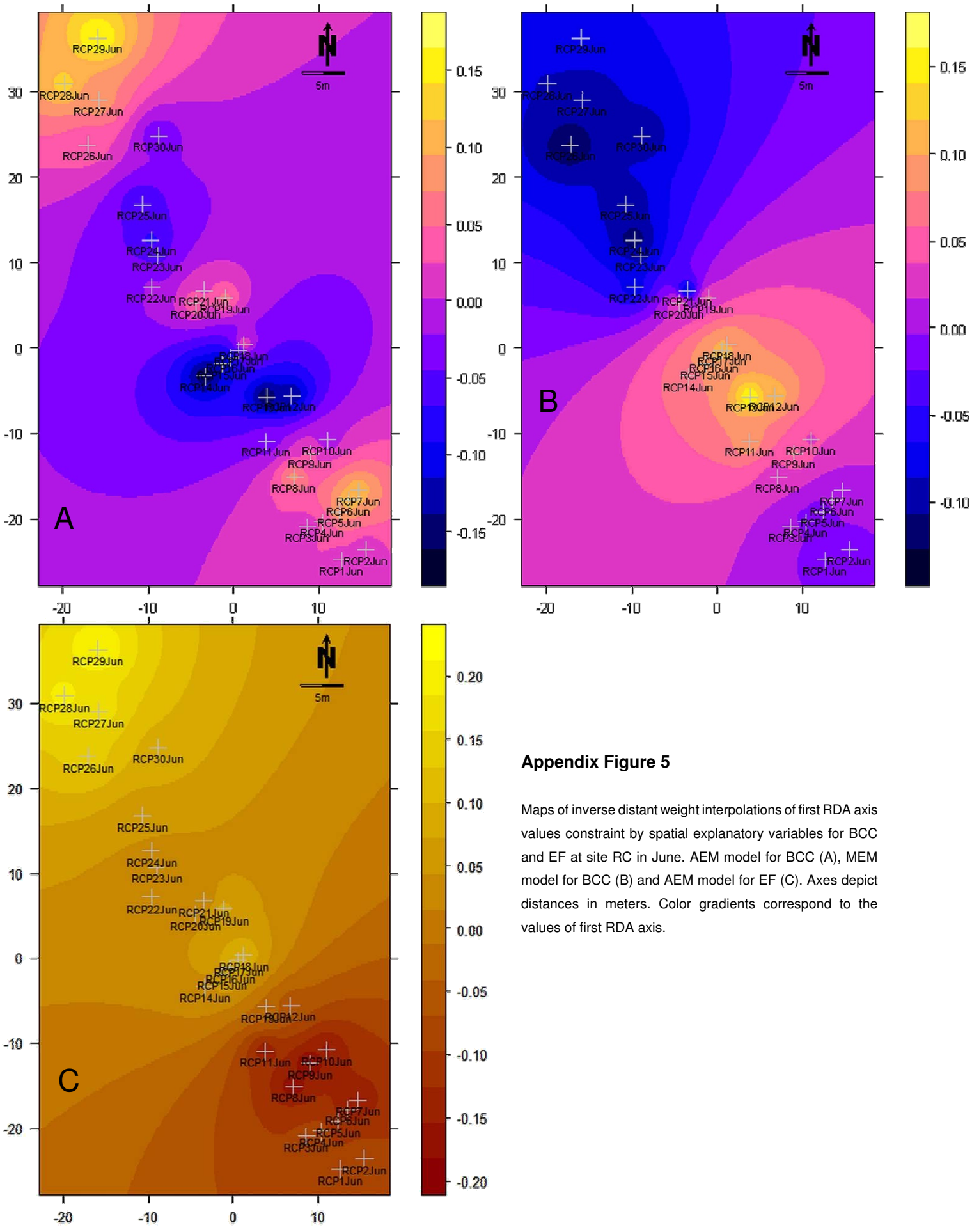
Appendix Figure 3

NMDS of the ARISA profiles of all seasons and sampling sites. The size of the dots is relative to the number of OTUs detected. Colors correspond to the relative system (SC and RC) and the locations of the piezometers (in-stream, riparian zone). Dispersion-ellipses depict the standard error of weighted average scores of groups split by systems and locations (confidence limit = 0.95). Enzymatic activities are fitted as arrows. Vectors fitted with $P<0.05$ are depicted in blue.



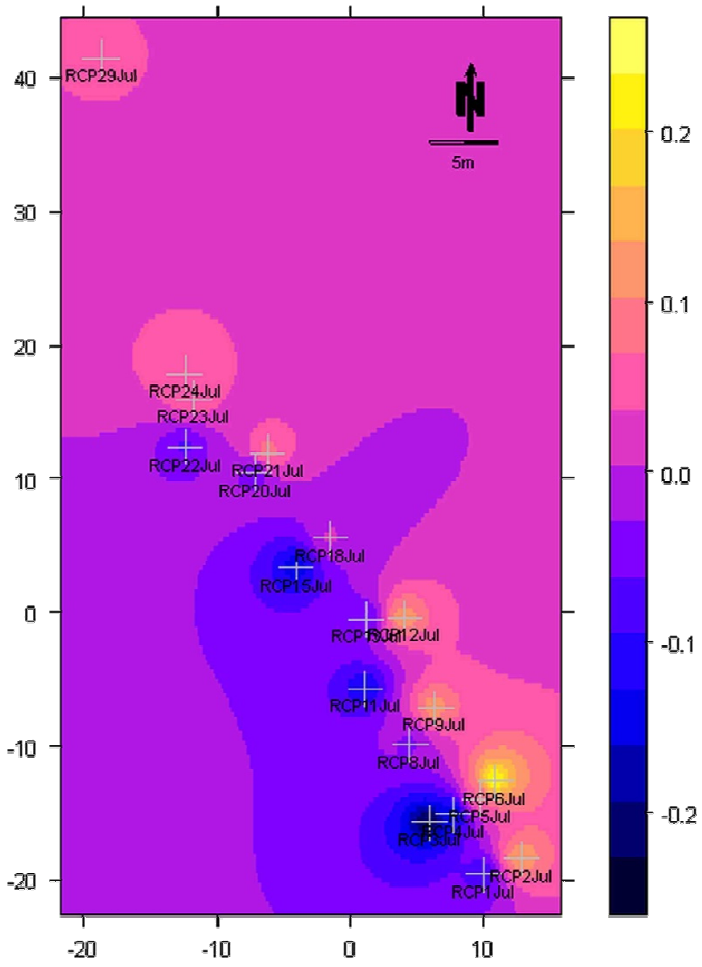
Appendix Figure 4

NMDS of the enzymatic activity profiles of all seasons and sampling sites. Enzyme scores are annotated. The size of the dots is relative to the square root of the sum logarithms of the measured enzymatic activities. Colors correspond to the relative system (SC and RC) and the locations of the piezometers (in-stream, riparian zone). Dispersion-ellipses depict the standard error of weighted average scores of groups split by systems and locations (confidence limit = 0.95). OTUs are fitted as arrows. Vectors fitted with P<0.001 and P<0.01 are depicted in blue and grey respectively.



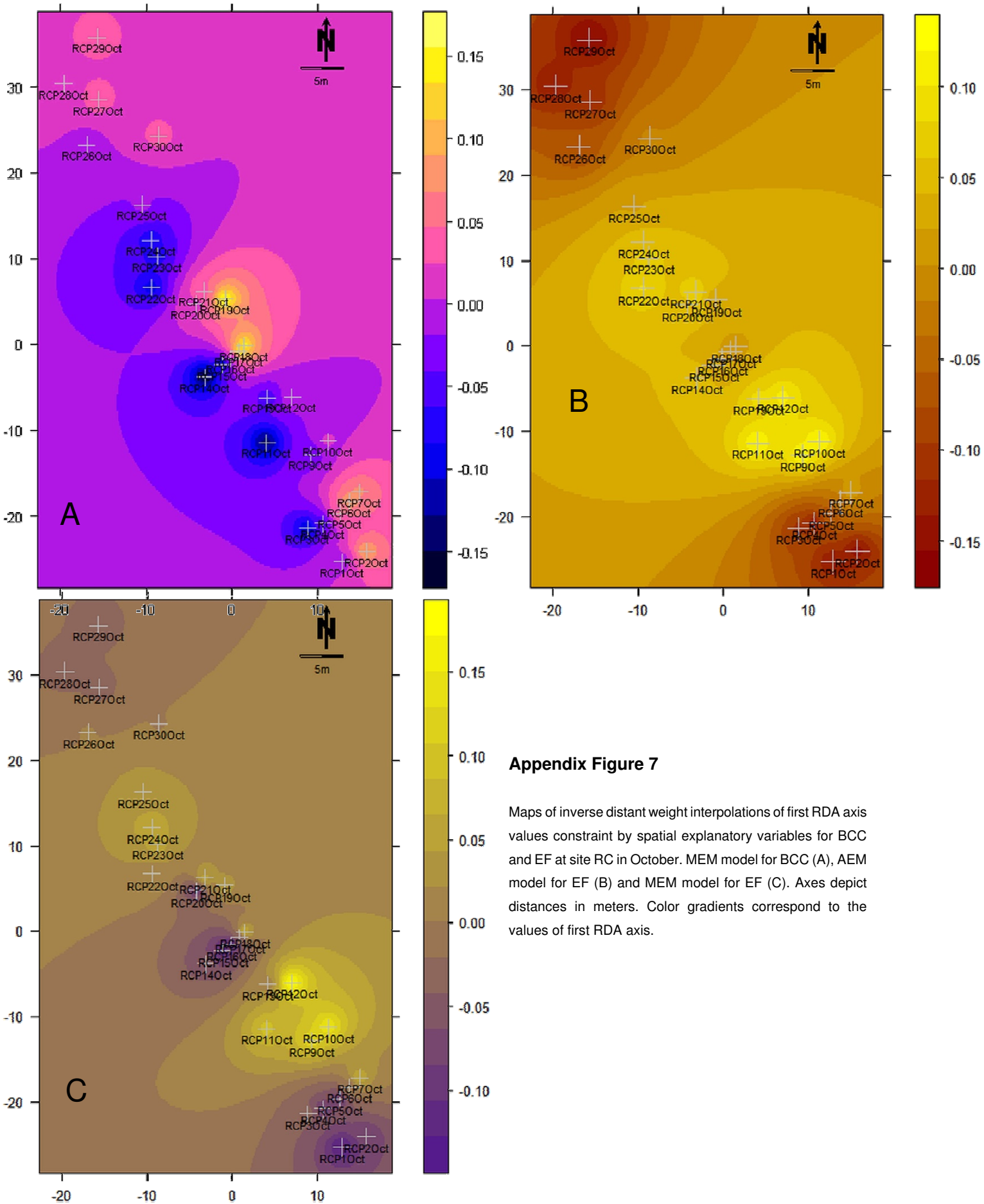
Appendix Figure 5

Maps of inverse distant weight interpolations of first RDA axis values constraint by spatial explanatory variables for BCC and EF at site RC in June. AEM model for BCC (A), MEM model for BCC (B) and AEM model for EF (C). Axes depict distances in meters. Color gradients correspond to the values of first RDA axis.



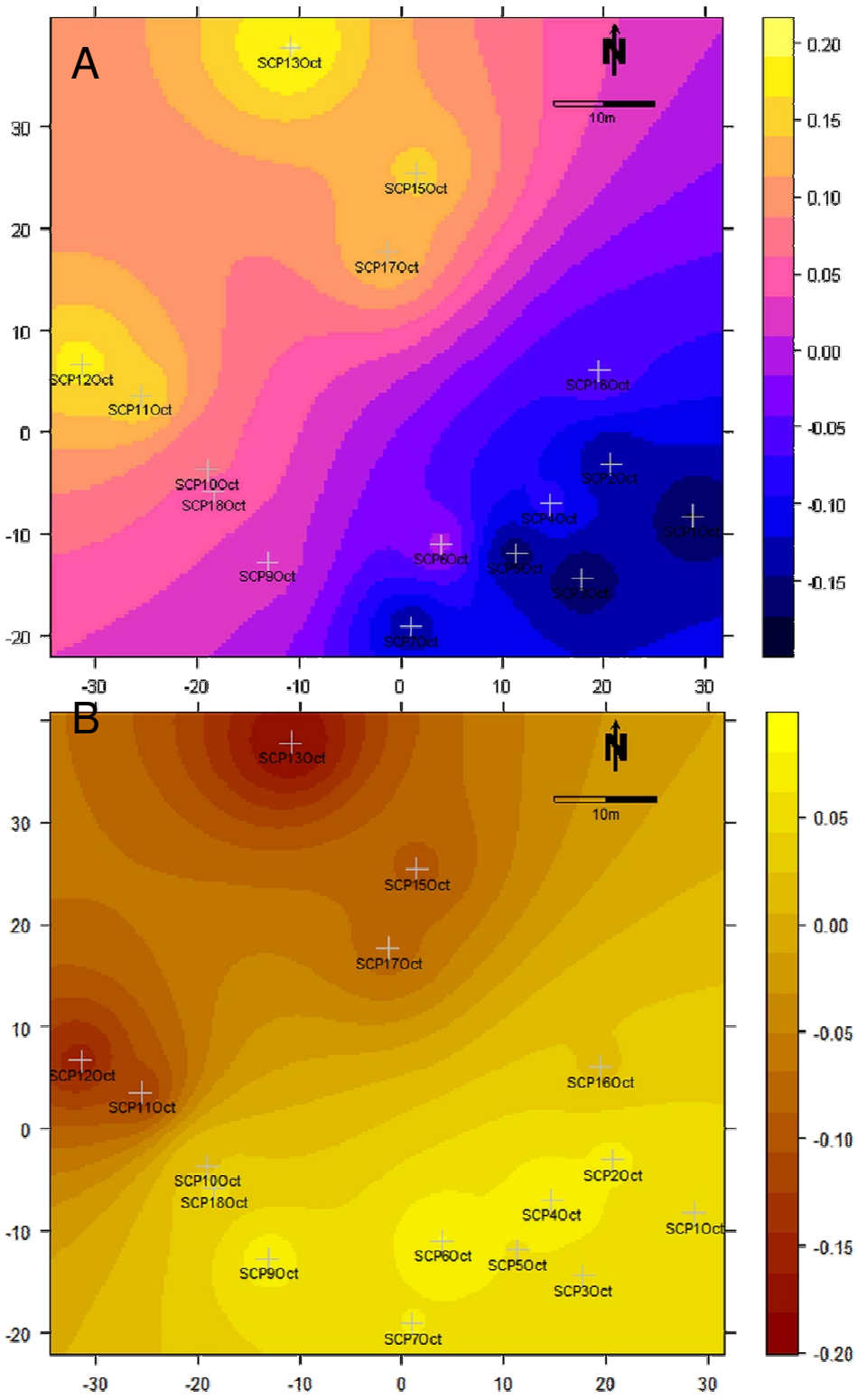
Appendix Figure 6

Map of inverse distant weight interpolations of first RDA axis values constraint by MEM variables for BCC site RC in July. Axes depict distances in meters. Color gradients correspond to the values of first RDA axis.



Appendix Figure 7

Maps of inverse distant weight interpolations of first RDA axis values constraint by spatial explanatory variables for BCC and EF at site RC in October. MEM model for BCC (A), AEM model for EF (B) and MEM model for EF (C). Axes depict distances in meters. Color gradients correspond to the values of first RDA axis.



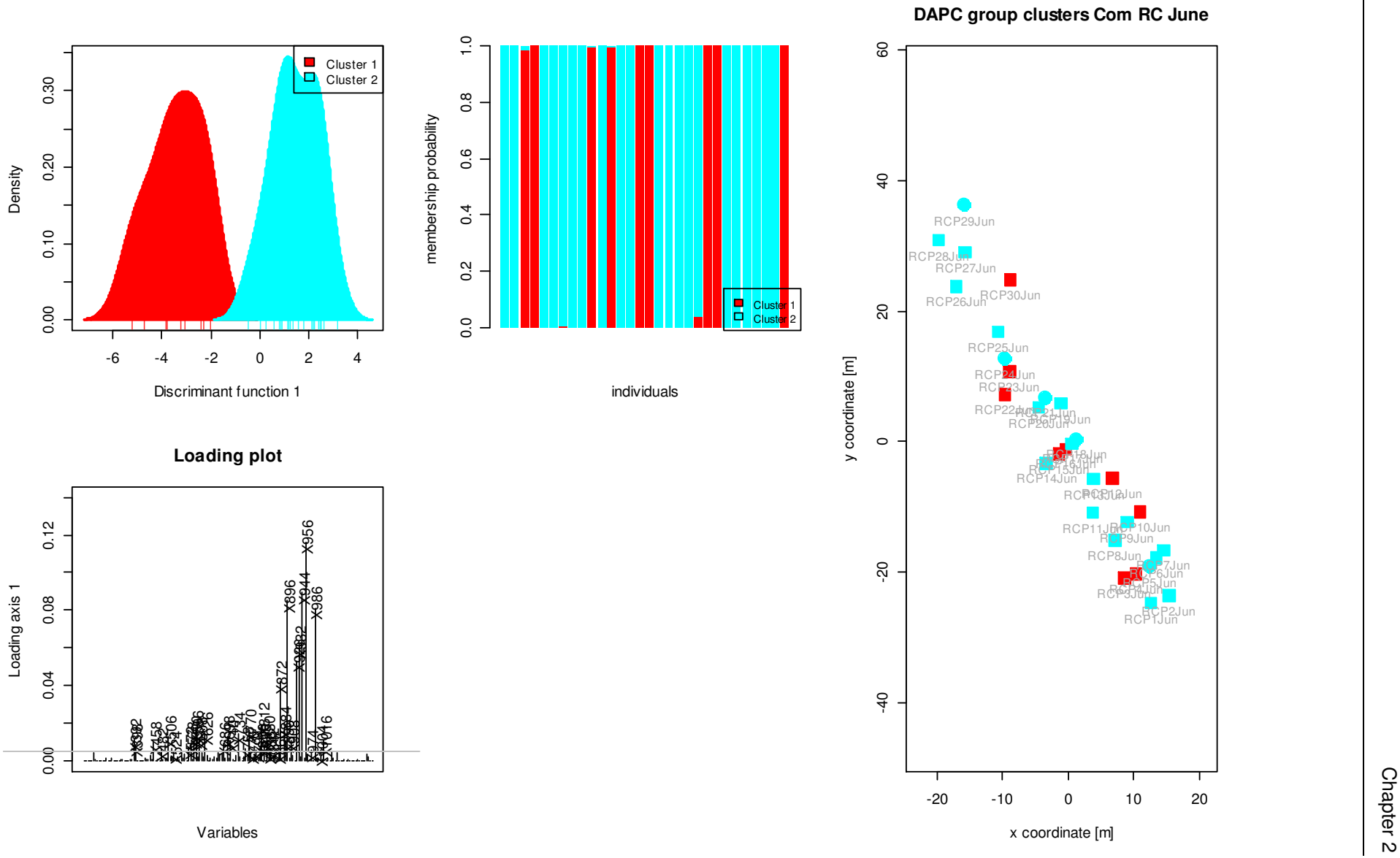
Appendix Figure 8

Maps of inverse distant weight interpolations of first RDA axis values constraint by spatial explanatory variables for BCC and EF at site SC in October. AEM models for BCC (A) and EF (B). Axes depict distances in meters. Color gradients correspond to the values of first RDA axis.

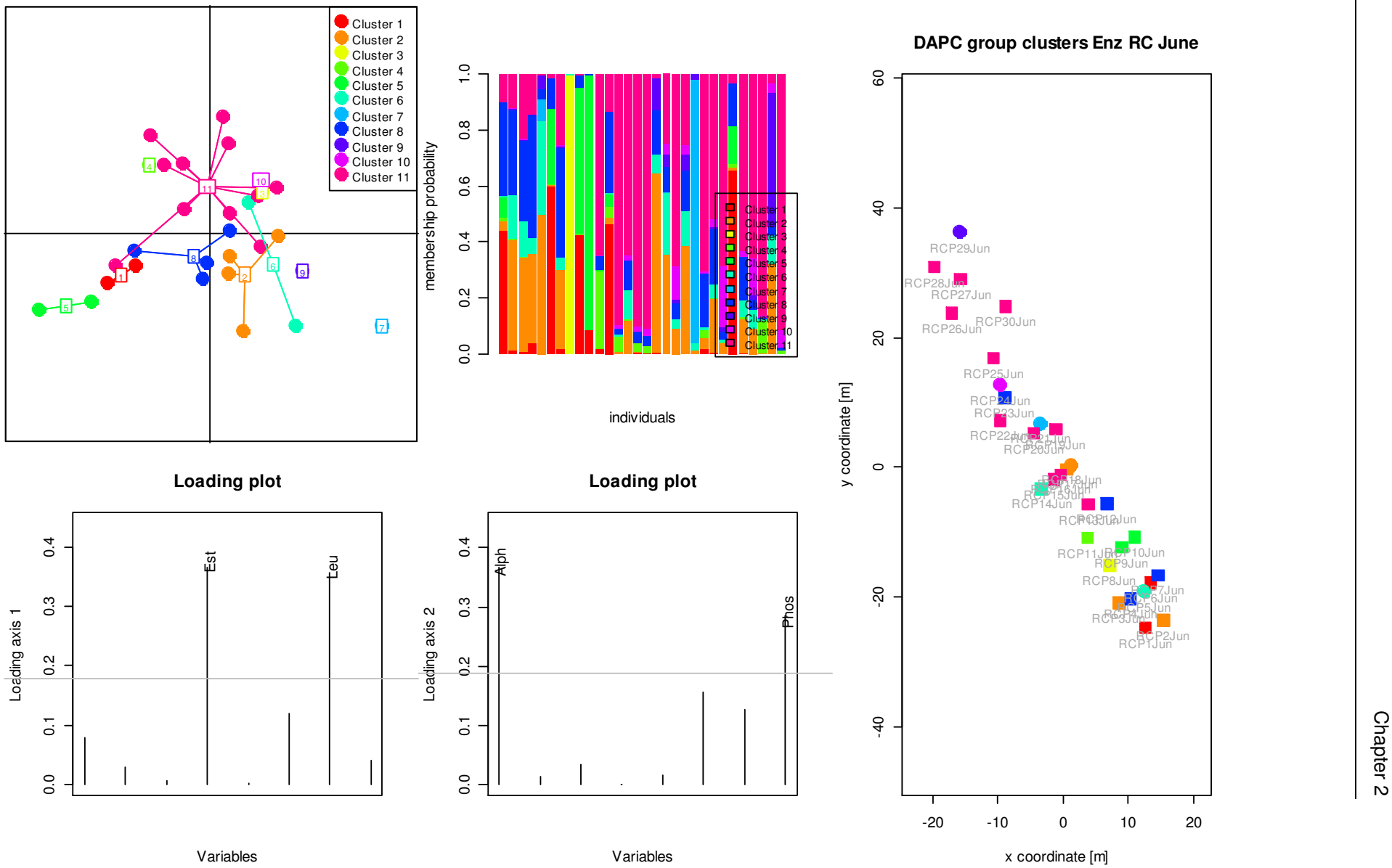
Appendix Figures 9A to 13B

Results from the DAPC analysis for the BCC (Figures a) and the EF (Figures b). Upper pannel shows the density curve of the firts discriminant function or the scatterplot of the first two discriminant functions of the DAPC. A priori defined clusters are shown by different colours and annotated with the respective cluster. Assignment probabilities of the single piezometers BCC and EF, respectively, are given on the right hand from the DAPC clustering. The ordering is increasing with the relative piezometer identification. Lower Pannel depicts the loading plots for the firs and second axis of the DAPC respectively. Annotated are the variables above the thresholdlevel of the third quartile of variables. The right hand panel depicts the geographic position of the piezometers. Defined clusters are depicted with corresponding colors. Figure 9 to 11 depict the RC floodplain in June, August and October respectively. Figure 12 shows the SC floodplain in October.

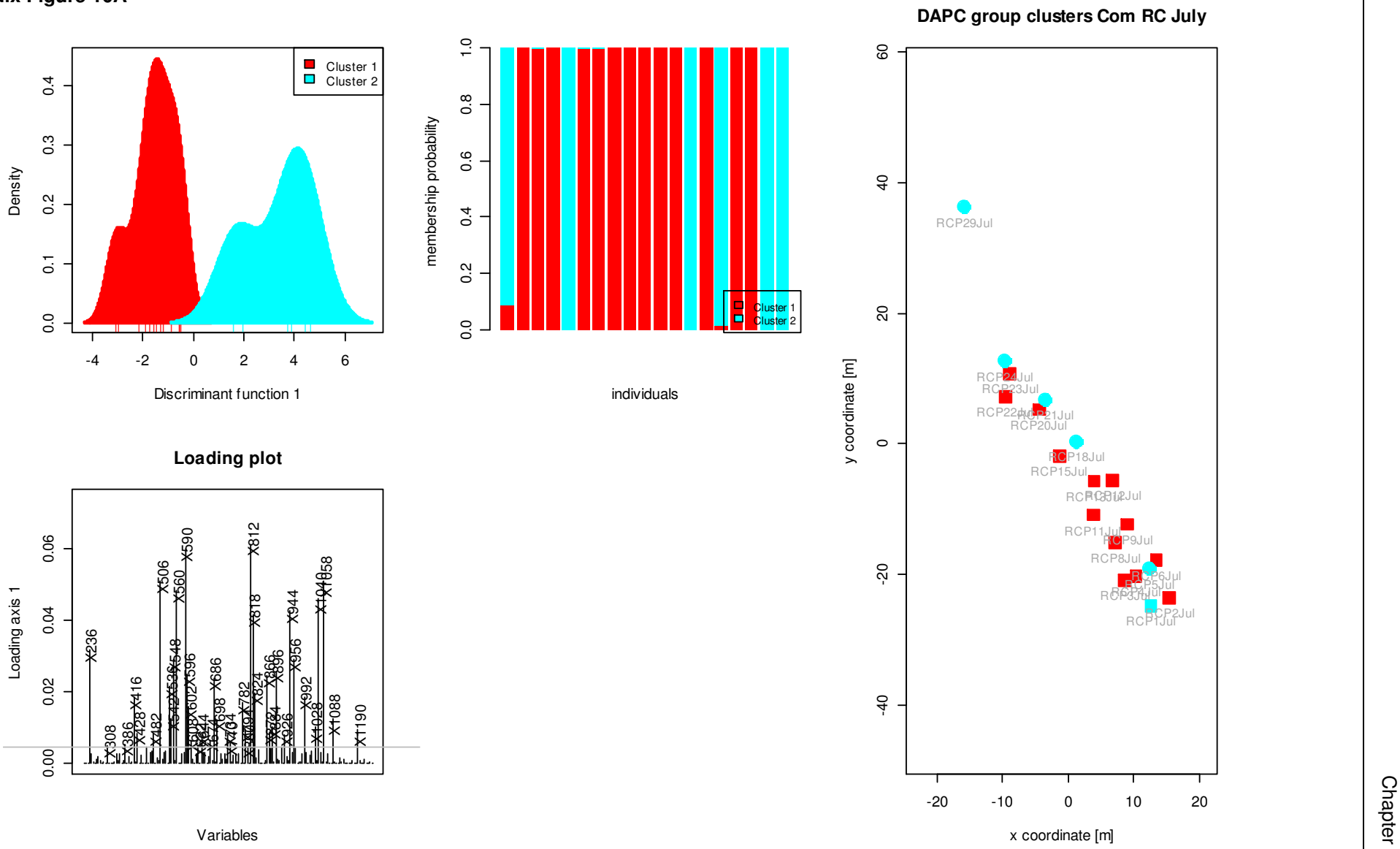
Appendix Figure 9A



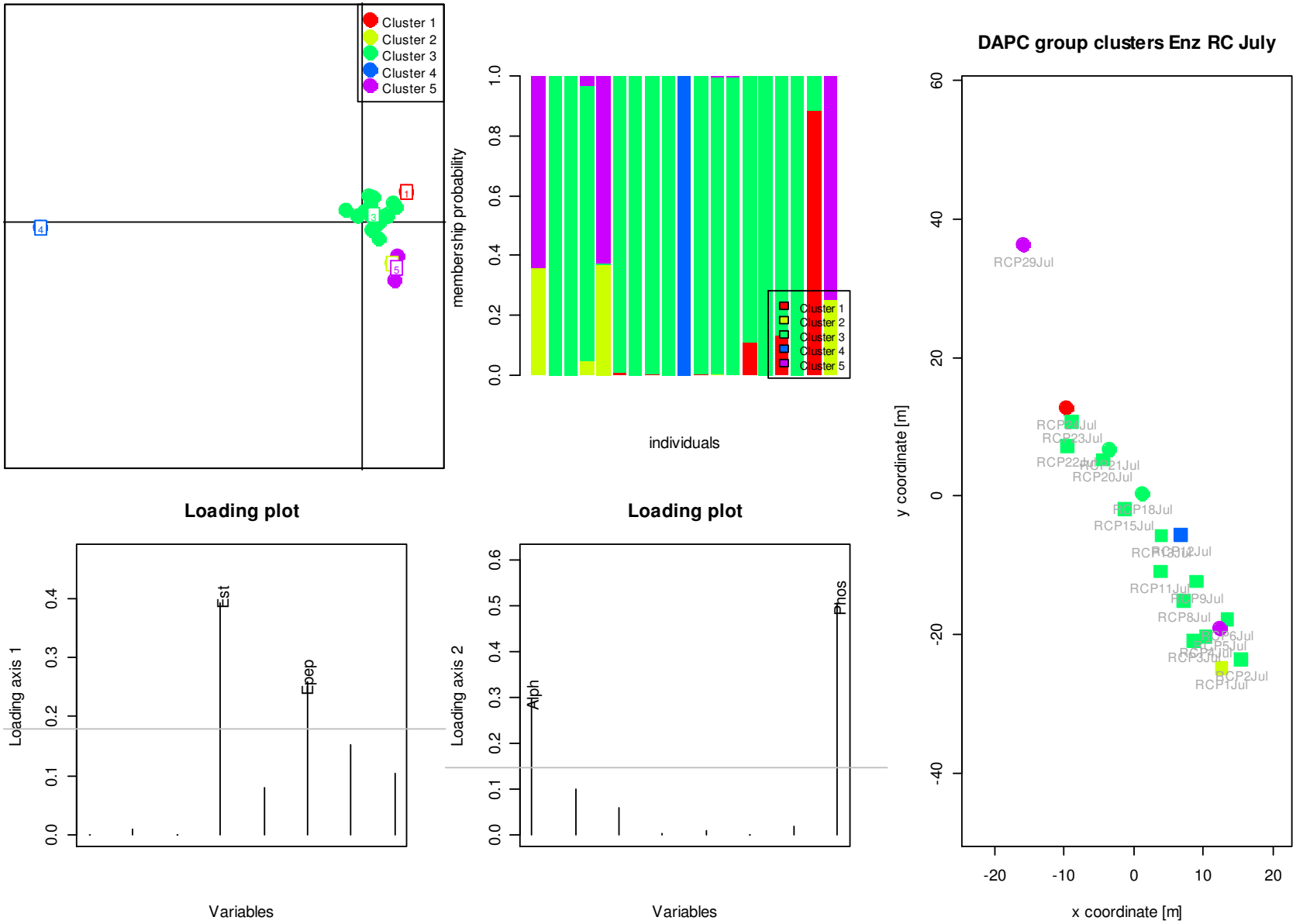
Appendix Figure 9B



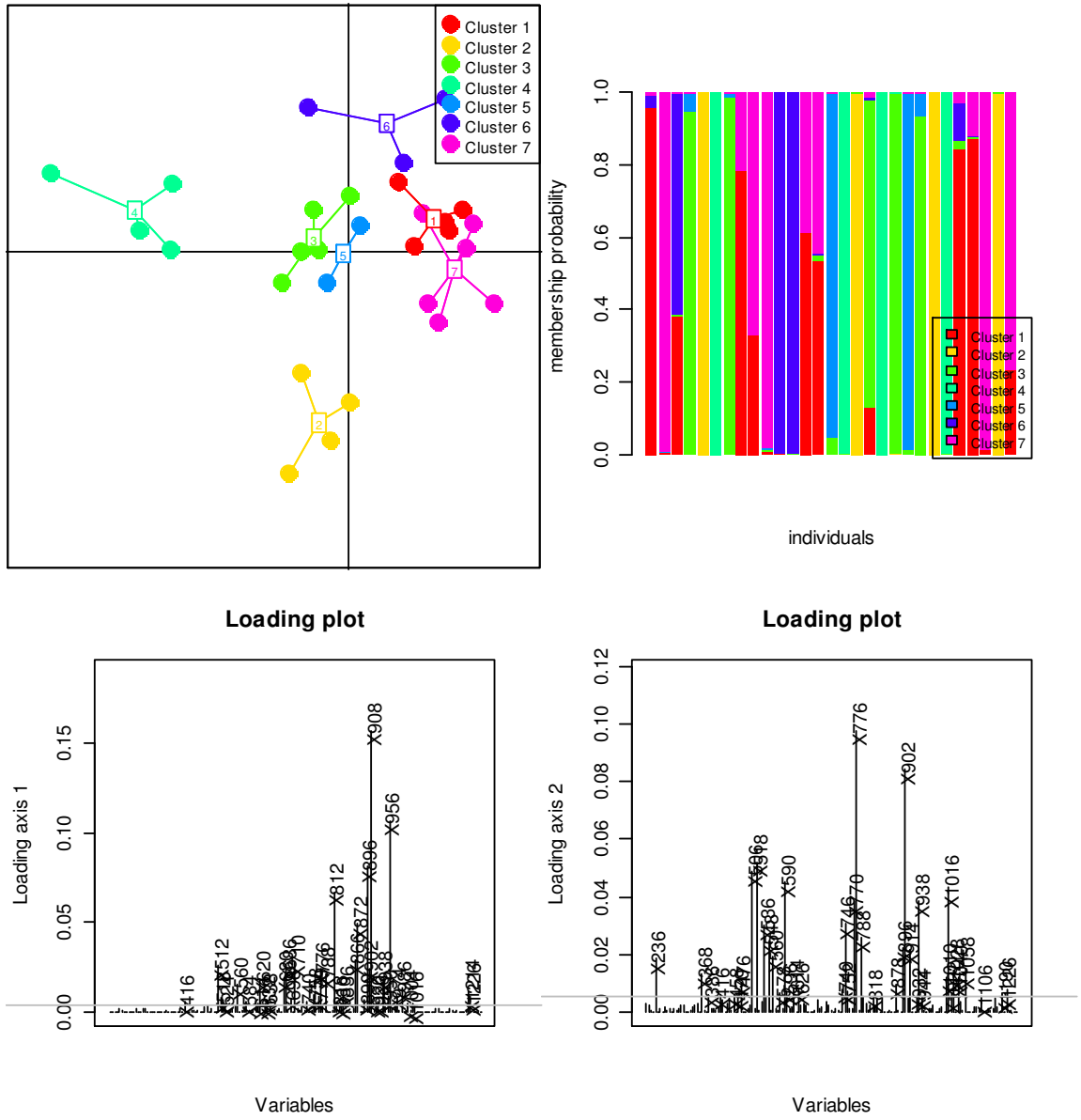
Appendix Figure 10A



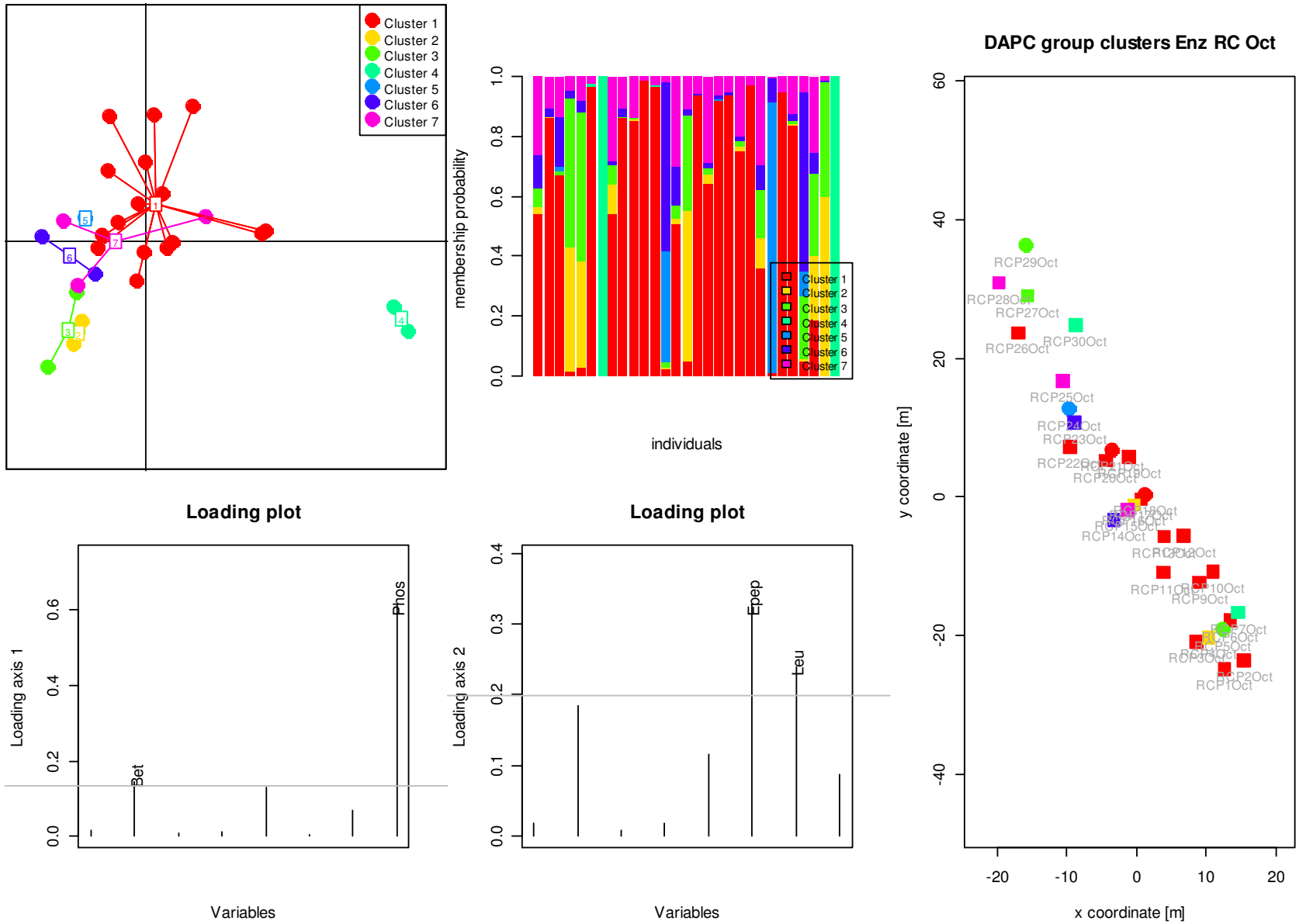
Appendix Figure 10B



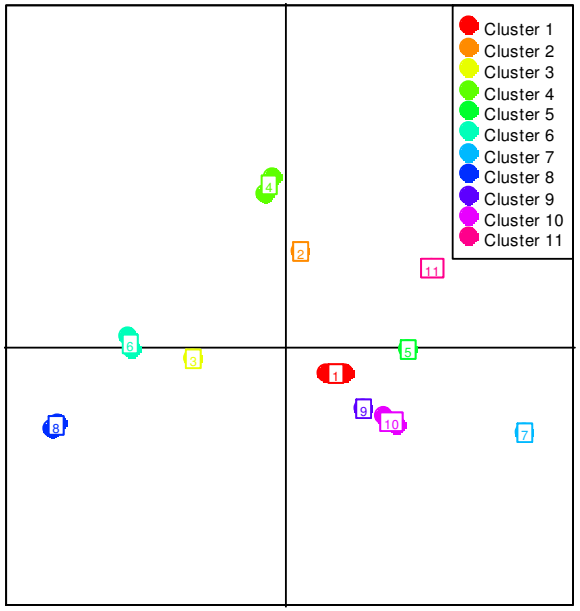
Appendix Figure 11A

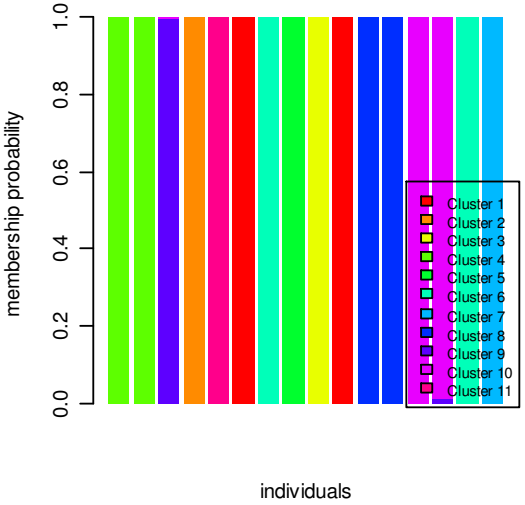


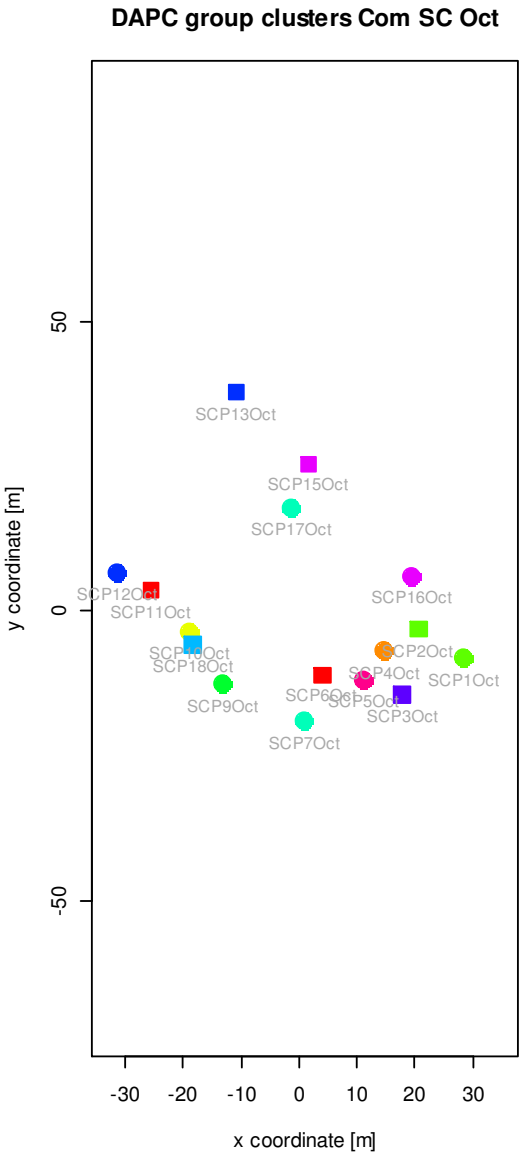
Appendix Figure 11B

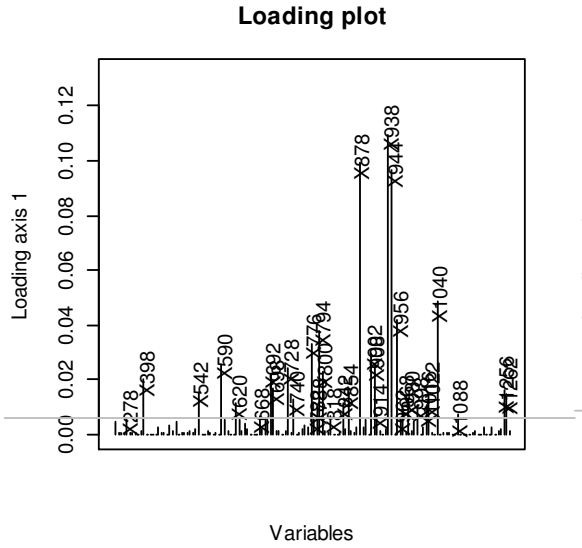


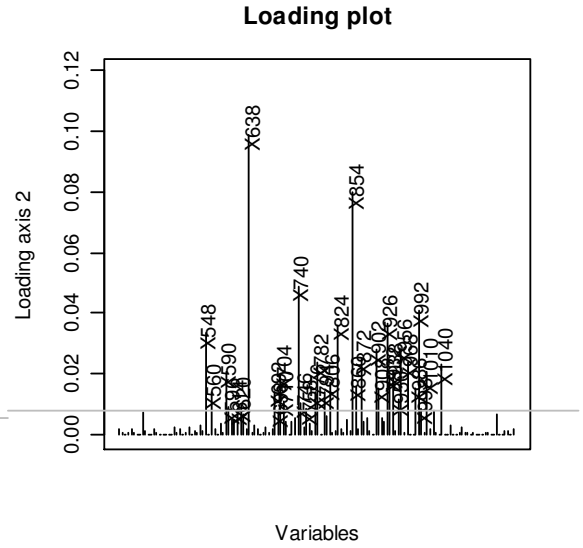
Appendix Figure 12A



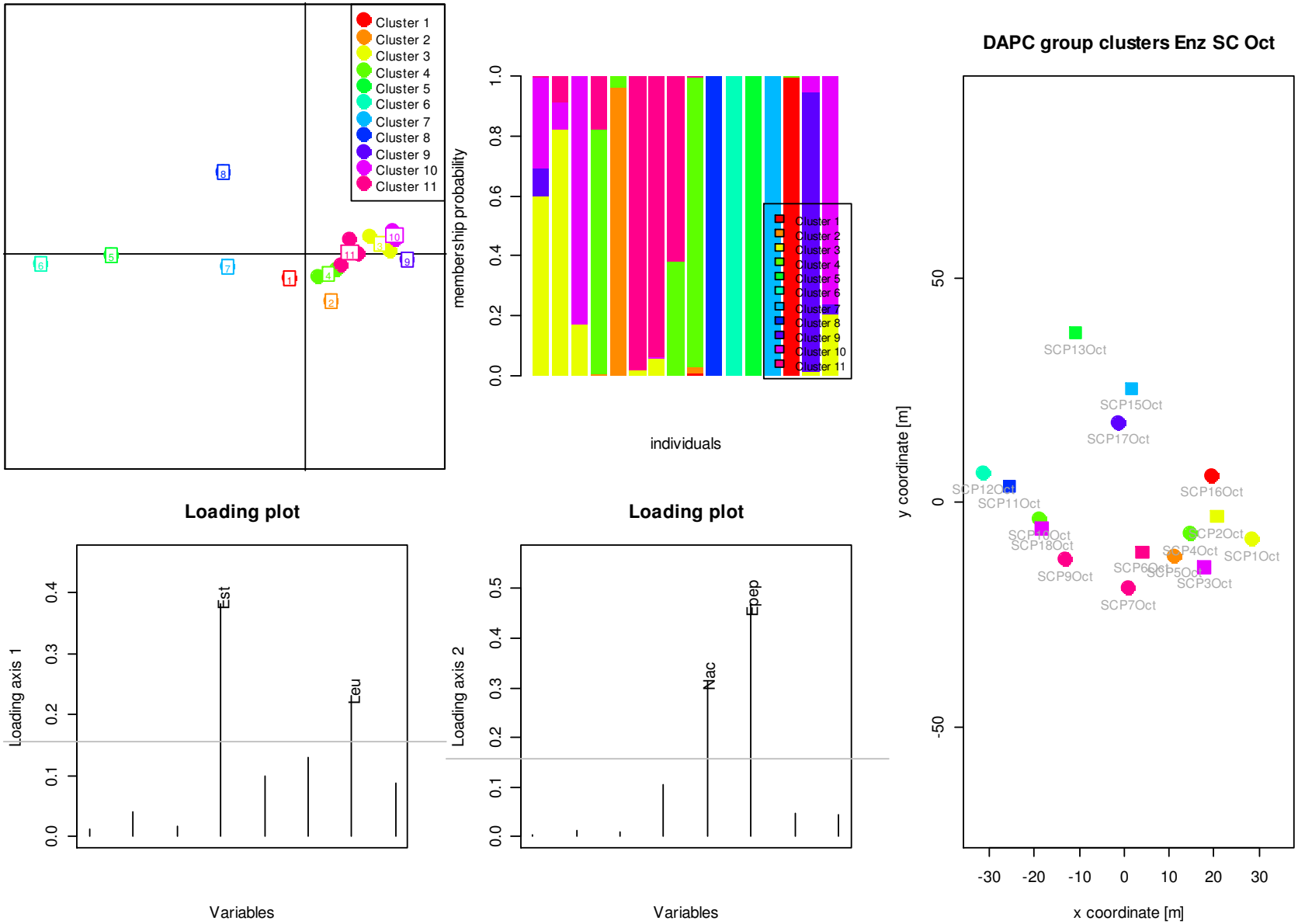








Appendix Figure 12B



Paper 3

Response of Lotic Microbial Communities to altered Water Source and Nutritional State in a Glaciated Alpine Floodplain

Response of Lotic Microbial Communities to altered Water Source and Nutritional State in a Glaciated Alpine Floodplain

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Under review in *Limnology and Oceanography*

Running Head: Alpine stream microbial communities

Keywords

Mesocosm / Alpine Floodplain /Glacial Retreat / Bacteria / Enzyme / ARISA / Microbes / Community Composition

Abstract

Microbes play a key role in the functioning of glaciated alpine lotic ecosystems. Factors driving bacterial community composition (BCC) and linkage to ecosystem function (EF) are a fundamental interest in microbial ecology. Climate warming is expected to result in a shift in water regimes from glacial (kryal) water to groundwater (krenal) dominated catchments due to receding glaciers, which is likely accompanied by a shift in BCC and EF. In this context, we performed a reciprocal transplant experiment of hyporheic sediments within a Swiss alpine floodplain. We assessed the influence of water source (krenal, kryal) and nutritional state (C, N, and P) on BCC and EF. Experimental response was tested using automated ribosomal intergenic spacer amplification (ARISA) and potential activities of 8 different enzymes. We found that BCC in both kryal and krenal systems were highly resistant to disturbance yet exhibited pronounced EF flexibility. Major factors determining BCC and EF were the origin of sediments followed by seasonal variation. The gradient in seasonal changes in BCC showed different strengths in the two water systems. Krenal BCC was generally more stable compared to the kryal one, although functional plasticity showed the same extent in both. This difference in connectivity between BCC and EF suggests that krenal BCC is dominated by generalists, whereas kryal systems are dominated by specialists. The weak effect of altered nutritional state on BCC and EF suggests a highly complex but hierarchically structured relationship among these factors. Our results suggest that microbial communities in alpine catchments may be able to rapidly buffer the impact of shifts in water source on ecosystem functioning.

Introduction

Most alpine glaciers have been retreating over the last decades due to climate change (IPCC 2007), and proglacial floodplain ecosystems are highly influenced by this glacial recession. Landscape characteristics such as hydrology, hydrochemistry, river channel expansion and retraction, riparian vegetation and channel stability also are expected to undergo dramatic changes (Milner *et al.,* 2009). These changes within catchments depend strongly on local situations such as the rate of glacial recession, altitude (e.g. tree line, temperature) and on the strength of interactions of the above-mentioned factors (Gurnell *et al.,* 2000).

A major feature of environmental conditions in alpine ecosystems is linked to the type of water source. Future predictions based on climate models suggest that glacial (kryal) water will become temporarily more important as long there is strong glacial mass loss occurring (Gurnell *et al.,* 2000). Over longer time scales however, it is likely that the importance of kryal water as a habitat shaping agent of these floodplains will diminish, followed by greater contributions by groundwater (krenal water) and precipitation runoff (Uehlinger *et al.,* 2010). These water sources are distinct in their physico-chemical properties and their seasonal dynamics. Habitat heterogeneity (physical and chemical) and the extent in hydrological connectivity, e.g. between channels or between channels and the riparian zone, are expected to change in concert with shifting water sources (Ward *et al.,* 1998).

Climate change and anthropogenic influences also are likely to alter the nutritional state and hydrology of alpine surface waters: e.g. atmospheric deposition is a primary contributor to the annual nitrogen (N) input into these systems (Hiltbrunner *et al.,* 2005). As deposition levels increase and the timing of nitrogen release is strongly coupled to the seasonal storage in snow packs, an elevated but less seasonally pronounced nitrogen input can be expected (Burns 2003, Hiltbrunner *et al.,* 2005). In addition, phosphorous (P) plays an important role within alpine ecosystems and potentially represents the limiting nutrient for heterotrophic growth, primary production, and decomposition. Glacial run-off as well as the hydrologic linkage of stream sediments with the surrounding soils and their characteristics influence the availability and forms of P and may be changing as well (Tockner *et al.,* 1997, Brady and Weil 2008). Vegetation also is sensitive to elevated temperatures, CO_2 and altered precipitation patterns and are changing and likely to change further within alpine regions (Theurillat and Guisan 2001). This vegetative change can influence organic matter (OM) input (quality and quantity) into glaciated floodplains (Zah and Uehlinger 2001). Additionally, in-stream primary production and respiration could be modulated by elevated temperature and CO_2 (Yvon-Durocher *et al.,* 2010).

Microbes such as heterotrophic bacteria play a major ecological role in most ecosystems. This role is not only due to the sheer abundance and biomass they represent, but also due to their ability to carry out a whole range of ecosystem processes. The hyporheic zone is an ecotone of particular interest within proglacial floodplains because it is the interface where surface water/groundwater interactions occur. Bacteria are the primary drivers of ecosystem processes occurring within this zone (Naegeli and Uehlinger 1997, Malard *et al.,* 2000b, Findlay 2010). This zone represents the interface between surface- and groundwaters, where nutrient exchange and cycling occurs and where water and nutrient flows create connectivity between riparian and alluvial zones (Findlay 1995, Boulton *et al.,* 1998).

We expect strong differences across seasons and habitats in both bacterial community composition (BCC) and ecosystem functionality (EF) mediated by bacteria in hyporheic stream sediments (Feris *et al.,* 2003). This variability is primarily driven by the formation of heterogeneous patches due to spatio-temporal differences in environmental conditions acting as drivers of BCC. Differential BCC and environmental properties then provide the template for bacteria to perform different metabolic activities (Winemiller *et al.,* 2010). OM input of vegetation, nutrient pulses during snow melt, hydrologic connectivity patterns (longitudinal, vertical and lateral flows), and the flow regime (e.g. amount of glacial melt water) drive stream heterogeneity (Stanford and Ward 1988, Battin 2000, Feris *et al.,* 2003, Battin *et al.,* 2004, Logue *et al.,* 2004, Poole *et al.,* 2006, Lowell *et al.,* 2009, Augspurger *et al.,* 2010).

Our study assessed the impact and strength of interactions of shifting water source and different BCC on EF within a proglacial floodplain. We conducted a reciprocal transplant experiment of hyporheic sediments from a krenal and kryal channel in combination with nutrient amendments (C, N+P, and C+N+P). The experiment was repeated during three different seasons to cover temporal changes in landscape properties on microbial assemblages and functioning in both water systems. We hypothesized that the different water sources and associated seasonal fluctuations in chemistry would drive BCC and EF. Moreover, we expected to see a response in BCC and EF due to nutrient amendment.

Methods

Study site

Two streams (kryal, krenal) were chosen within the Val Roseg catchment in the Swiss Alps to perform the sediment transplant experiment. The catchment is part of the austroalpine Bernina nappe and consists of crystalline bedrock (Malard 1999). Around 30% of the catchment area is glaciated by the Roseg and Tschierva glaciers (BAFU 2010). The Roseg River is an 11.3-km long second order tributary of the river Inn and represents the main channel within this glacial floodplain. Its discharge regime is mainly dictated by water originating from the two glaciers; ca. 30% of the annual water volume is glacial meltwater. This contribution is higher during the glacial ablation period from spring until autumn and decreases during winter. The Roseg River gains lateral inputs from groundwater-fed tributaries that show distinct seasonal inputs from snow melt. The relative importance of this water source increases within the floodplain when glacial ablation is low during winter (Tockner *et al.,* 1997). The glacial stream site (kryal) was situated within the floodplain (Site g, N 46°25''18', E 9°51''40') and the groundwater fed stream (krenal) was situated next to the valley side (Site r, N46°25''13', E9°51''41').

Study design and sampling

A fully factorial reciprocal transplant experiment with sediment in flow-through mesocosms was performed, taking into account two sediment origins (SO; Krenal or Kryal), two incubation sites (IS; Krenal or Kryal), and four treatments (T; zero control (Z), C, NP, CNP; see below). The experiment was replicated across three seasons (S; Summer (A), Winter (O), Spring (M)) (Figure 1). At each site (Krenal or Kryal), stream hyporheic sediment was collected after removing the top 10 cm and an <8 mm (by sieving) size fraction was filled into an open-ended double-chambered mesocosm tube (6.5 cm diameter, 30 cm long) with both ends capped with 1-mm mesh nylon screen. The second chamber was separated by a 1-mm mesh and kept empty (zero treatment) or used for three different nutrient amendments: carbon (C), nitrogen (N) and phosphorous (P) or C, N, and P together. Osmocote Exact slow release fertilizer tablets (Hauert HBG Dünger AG, Grossaffoltern) were used to add nitrogen as nitrate (NO_3-N) and ammonium (NH_4-N) (7.5 % w w^{-1}, each), and phosphorous expressed as water soluble phosphorus pentoxide (P_2O_5) (10% w w^{-1}). Two tablets were used per treatment (~10g). Potassium acetate (Sigma-Aldrich Co, P1147) was used for C amendment, and 36 g potassium acetate (8.82 g C) was dissolved with stream water in a 50-ml Falcon tube and placed within the respective mesocosm. To ensure a constant release during the incubation period, an osmotic membrane was used to seal the Falcon tube (Spectra Por® Type F, Spectrum Laboratories, 250 kDa).

In the field, mesocosms were inserted with the nutrient supply chamber facing the water flow. Three mesocosms per treatment were either embedded within the sediment source stream or transplanted into the non-native water fed stream (Figure 1). Preliminary tests confirmed the slow release properties of the nutrient sources. Osmocote tablets showed an average weight loss of 0.15±0.02 g d^{-1}. Potassium acetate had a linear release rate when tested in the lab (-0.0189 g C h^{-1}, R^2=0.93) and showed an average release of 0.24±0.09 g C d^{-1} in the field experiments as determined by the total carbon left in the Falcon tubes after the incubation period. Taking into account flow velocity within the boundary layer of the streams (~0.1 m s^{-1}) and an approximately 20-fold decrease in water velocity within the packed and submerged mesocosm sediment, one can expect an increase of C (compared to dissolved organic carbon (DOC) concentrations) of around 40% and 35% compared to averaged background concentrations of krenal and kryal waters, respectively. NH_4-N would show an increased concentration of 400% and 200%, whereas NO_3-N concentration would increase about 10% and 5% in krenal and kryal streams, respectively. P_2O_5-P would increase P concentrations around 200% and 150% for krenal and kryal streams, respectively, compared to background phosphate (PO_4-P) concentrations. These numbers are just estimates because we do not know the exact flow rate of the water within each mesocosm.

Incubations were performed in August and October 2009, and May 2010 for 21 days each. At the end of incubation, sediments were collected and either directly processed for microscopic examination or frozen at -20°C for automated ribosomal intergenic spacer amplification (ARISA) and assessment of potential enzymatic activities. Freezing is thought to have only minor storage effects on enzyme activities or bacterial community profiling (Wallenius *et al.,* 2010).

Specific conductivity (µS cm^{-1} at 20°C) and temperature were measured with a conductivity meter (LF323, WTW, Weilheim, Germany). Surface water samples (1 L) were collected and transported in a cooling box to the laboratory. The water was then filtered through pre-ashed glass fiber filters (GF/F, Whatmann) and the filtrate analyzed for dissolved organic carbon (DOC), particulate

organic carbon (POC), total inorganic carbon (TIC), ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N), dissolved organic nitrogen (DN), particulate nitrogen (PN), phosphate (PO_4-P), dissolved phosphorus (DP) and particulate phosphorus (PP) following standard protocols (Tockner *et al.,* 1997).

Bacterial cell numbers

A 0.5 ml aliquot of collected sediment was suspended in 1.11 ml paraformaldehyde (2%, final concentration) in an Eppendorf tube and fixed for 24 h at 4°C followed by three washing steps with 1 x PBS and 5 min centrifugation at 10,000 g between washing steps. Samples were then stored at -20°C in a 1:1 mix of PBS/ethanol until further processing (Pernthaler *et al.,* 2001). Biofilm-associated bacteria were detached with sonication (Branson Digital Sonifier 250, Danbury, USA, 5-mm tapered microtip, actual output of 20 W) using 1-s sonication pulses for 30 s. Samples were vortexed for 7 s followed by a short spin centrifugation for 5 s to settle coarse sediment particles interfering with subsequent processing of samples. The supernatant was transferred into a new Eppendorf tube and used for total cell counting of 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich Co) stained cells. Between 10 to 60 µl of template solution was pipetted into 5 ml sterile water (MQ) and stained with DAPI for 7 min (1 µg ml^{-1} final concentration) then filtered onto a black polycarbonate filter by applying a gentle vacuum (0.2-µm pore size, 25-mm diameter, Millipore, Molsheim, GTBP02500) (Porter and Feig 1980). Filters were embedded into citifluor AF1 after air drying (Linaris Biologische Produkte, Wertheim, Bettingen). A minimum of 16 photographs of each filter was taken with an epifluorescence microscope (Leica Microsystem, DMI6000b) and a minimum of 800 cells were counted per filter using the CellC software (Selinummi *et al.,* 2005). Cell numbers were then standardized to the dry mass of initially suspended sediments.

Enzyme assays

Eight enzymes were tested for their potential activity using Methylumbelliferone (MUF)-labeled substrate analogues (Sigma-Aldrich CO). Enzymes degrading polysaccharides were tested using 4-MUF-α-D-glucoside for Alpha glucosidase (*Alph*) which hydrolyses α-1,4- and 1,6-glucosidic linkages of polysaccharides, 4-MUF-β-D-glucoside for Beta glucosidase (*Bet*) which hydrolyzes beta-1,4-glucans, and 4-MUF-β-D-xylopyranoide for β-xylosidase (*Xyl*) which hydrolyses xylose residues. 4-MUF-N-acetyl-β-D-glucosaminidase was used to assess hydrolysis of 1,4-β-linkages of glucosamines by N-acetyl-glucosaminidase (*Nac*) (Sinsabaugh *et al.,* 2008). Esterase (*Est*) activity was measured using 4-MUF-acetate (Arpigny and Jaeger 1999). Leucine aminopeptidase (*Leu*) and endopeptidase (*Epep*) activity were measured using L-leucine-7-amido-4-methylcoumarin and 4-MUF-4-guanadinoenzoate, respectively (Vihinen and Mäntsälä 1989, Makoi and Ndakidemi 2008). 4-MUF-phosphate was used to assess phosphatase (*Phos*) activity.

Approximately 10 g of the frozen sediment sample was thawed over night at 4°C and subsequently resuspended in 10 ml MQ and vortexed for 1 min. Supernatant was transferred into a 96 well microplate and substrate stock solution was added to a final concentration of 400 µM (Findlay *et al.,* 2001). The remaining sediment and MQ were dried at 60°C for 48 h to measure the dry weight of measured sediments. Fluorimetric enzyme assays were performed after adding the substrate over 24 h using a microplate reader (Tecan Infinite® 200, Switzerland). The excitation wavelength was set at 365 nm and fluorescence emission was measured at 445 nm. Plates were stored on a plate shaker at 15°C between measurements. Fluorescence values were corrected for quenching by adding a known quantity of free MUF to the samples and pure MQ or MQ/bicarbonate buffer mix, respectively. Reaction rates were calculated using the linear part of the fluorescence reaction curve. Sub-samples of the collected sediments were air-dried at 50°C and total sediment organic matter (OM) was determined as ash free dry mass by combusting the samples at 450°C for 4 h. Potential enzyme activities were standardized to nmol substrate g^{-1} OM h^{-1}.

Bacterial community fingerprinting

Automated ribosomal intergenic spacer amplification (ARISA) was used to determine bacterial community structure (Fisher and Triplett 1999). Frozen samples were extracted using the PowerSoil DNA isolation Kit (MoBio, Carlsbad) following manufacturer's instructions. Fluorescein (6-FAM) labeled universal forward primer 1406f-6FAM (16S rRNA gene, 5'-FAM-TGYACACACCGCCCGT-3', Y=T,C) and the bacteria specific reverse primer 23Sr (5'-GGGTTBCCCCATTCRG-3', B=G,T,C, R=G,A) were used to amplify bacterial ribosomal intergenic spacers (Yannarell *et al.,* 2003). PCR was performed using a TProfessionalthermocycler (Biometra GmbH, Göttingen). A final reaction volume of 25 µl contained a mix of 1x GoTaq®Flexi buffer, 3 mM $MgCl_2$, 0.25 mM of each dNTP, 0.05 U $µl^{-1}$ of GoTaq®Flexi DNA Polymerase (Promega, Switzerland), 0.25 mg ml^{-1} bovine

serum albumin (Sigma-Aldrich, Switzerland), 0.4 µM of each primer (Microsynth, Switzerland) and 1 µl of template DNA (~ 1 ng) was amplified.

Initial denaturation for 2 min at 94°C was followed by primer annealing at 55°C for 35 s and extension of 2 min at 72°C. Subsequently, 29 cycles with denaturation for 35 s and annealing and extension as above were performed, followed by a final extension of 2 min at 72°C. PCR products were subjected to electrophoresis on a 2% agarose gel with a 100 bp ladder (Promega, Switzerland) and stained with ethidium bromide to verify successful amplification. ARISA fragments were processed as previously described (Bürgmann *et al.,* 2011). A 1-µl aliquot of PCR product was mixed with 9 µl of HiDi formamide and 0.5 µl Liz1200 size standard (Applied Biosystems, Germany) followed by denaturation on a PCR thermocycler for 3 min at 95°C. Subsequently, samples were placed on ice. Denaturing capillary electrophoresis of each fragment was performed on a 3130XL Capillary Genetic Analyzer (Applied Biosystems, Germany) equipped with a 50-cm capillary using POP-7 polymer. ARISA fragments between 200 and 1400 bp were analyzed with the Southern size-calling method and a background cut off level of 50 fluorescence units. Binning of peaks was done with the automatic and interactive binning R scripts (Ramette 2009). Relative fluorescence intensity of binned peaks data was exported for further analysis.

Data analysis

Effects of treatment, incubation site and sediment origin on single enzyme activities and total cell abundances were tested by repeated measures analysis of variance (ANOVAs). Treatment, incubation site and sediment origin were treated as fixed factors in the ANOVAs. Normality of residuals was assessed by performing a Shapiro Wilk's test and examining the QQ-plot of the residuals. Levene's test was used to check homogeneity of variance. If one of the assumptions was violated, data were transformed by ln(x+1). Percentage values were arcsine(sqrt) transformed prior to analysis. If an interaction with season and/or sediment origin was significant, we analyzed the data separately for the different sediment origins and seasons by using treatment and incubation site as fixed factors. When there was significant influence of treatment, we checked for distinct treatment effect with a Tukey's test. Enzymes were also tested with an ANOVA model including season and sediment origin followed by a Tukey's test.

Results from community fingerprinting and enzymatic activities were analyzed using non-metric multidimensional scaling (NMDS). Environmental data, experimental factors, single OTU's and enzymatic activities were used for vector and factor fitting and tested by permutation tests. Dispersion ellipses using standard error of the weighted average of scores were drawn for season and sediment origin x incubation site, respectively. The weighted correlation defines the direction of the principal axis of the ellipse (Oksanen *et al.,* 2011). Generalized additive models (GAM) were fitted for enzyme activities in the NMDS plots to visualize if there is a linearity of enzymatic factor fittings in the ARISA NMDS plots and to assess the relationship of the enzyme centroids to their surrounding within the EF NMDS (Bennion *et al.,* 2011, Oksanen *et al.,* 2011).

We performed multivariate homogeneity of group's dispersion (MHGD), an analogue of Levene's test for homogeneity of variance, to test if fingerprinting profiles and enzymatic activities were distinguished in their variability. Bray-Curtis dissimilarities of transformed and standardized datasets were reduced to principal coordinates (PcoA) and distances to defined group centroids were then calculated. Significance between group dispersions were assessed using a permutation test (999 permutations) (Anderson *et al.,* 2006).

Permutational multivariate analysis of variance (PERMANOVA) was used to assess the influence of treatment, incubation site and season on community and enzyme activity structures (Anderson 2001). A complete model with interactions of all factors was used to assess changes in community structure and function. Hierarchical structured models, where impact of incubation site was tested within one sediment origin, impact of incubation site within one sediment origin and within a season, and the impact of treatment additionally within a distinct incubation site, were used to refine analysis of changes of community structure and function that were likely to be covered by strong effects of sediment origin and season. A procrustes analysis of the corresponding NMDS ordinations of ARISA profile and enzyme activities was used to assess potential linkage between structure and function (Gower 1975, Digby and Kempton 1987). This leads to a m^2 statistic, which is a measure of congruence of the two ordinations. The procrustes correlation r was calculated as $r = \sqrt{1 - m^2}$. Configurations of NMDS were scaled to equal dispersion. The two configurations were tested for non-randomness by the means of permutations with the protest() function (Mardia *et al.,* 1979, Peres-Neto and Jackson 2001). NMDS, MHGD and PERMANOVA's were based on Bray-Curtis dissimilarity matrices calculated from the Wisconsin double standardized relative fluorescence intensity of ARISA profiles and the Wisconsin standardized

square-root enzyme activities (Bray and Curtis 1957). All analysis were done using the vegan package in R (R Development Core Team, 2011, Oksanen *et al.,* 2011).

Results

Bacterial abundance and biomass

Bacterial cell abundance was significantly affected by season with lowest cell densities during spring within kryal sediments compared to the other seasons (ANOVA: $F_{2,69}$=3.86, P<0.05, Tukey's HSD: P<0.05; see Table 1 and Appendix Table 1 for detailed ANOVA results). Krenal sediments showed increasing cell numbers from summer towards winter with maximum cell numbers in spring (ANOVA: $F_{2,69}$=18.76, P<0.001, Tukey's HSD: P<0.05). Krenal sediments had generally higher cell abundances than kryal sediments (mean±se: kryal=19.94x10^6±2.85x10^6 cells g^{-1} dw, krenal=9.78x10^7±8.00x10^6 cells g^{-1} dw, ANOVA: $F_{1,96}$=11.76, P<0.001).

The dependence of bacterial cell abundance on treatment varied with season and sediment type (interaction of treatment, season and sediment origin: ANOVA: $F_{6,96}$=49.95, P<0.001). Additionally, incubation site influenced the effect of treatments on bacterial abundances depending on season (interaction of treatment, incubation site and season: ANOVA: $F_{6,96}$=25.60, P<0.001). Native krenal sediments had decreased bacterial abundance when exposed to a nutrient treatment in addition to a change into the non-native water source during winter. In contrast, the kryal sediment showed increased cell numbers due to treatment after transplantation into the non-native water source in winter (Tukey's HSD: P<0.05, Appendix Table 1).

Sediment OM showed an interaction of incubation site, season, and sediment origin (ANOVA: $F_{2,9}$=7.01, P<0.05). Generally, OM was highest in krenal sediments (ANOVA: $F_{1,142}$=11.05, P<0.01).

Enzymatic activity

Detailed results of ANOVA are reported in Table 1. The total enzyme activity (sum of the 8 enzyme activities) showed an effect of incubation site with higher activities in the krenal stream (Tukey's HSD: P<0.01). Four enzymes showed a highly significant effect of season. The interaction term for season and sediment origin was likewise significant for 6 enzyme activities, and the interaction term for season and incubation site was significant for 3 enzymes. This result indicates a pronounced seasonality of EF that is modulated by the type of sediment and water source. Significant treatment effects were observed for three enzymes. Treatment effects were mainly interconnected with season and to a lesser extent with sediment origin.

Generally there was a higher activity of *Alph*, *Leu,* and *Bet* in the krenal sediments except for the summer season where *Bet* showed equally low activity in krenal sediment compared to kryal sediments in all seasons(ANOVA: $F_{1,138}$=30.09 and 17.71, P<0.001 and $F_{2,138}$=13.61, P<0.001, Tukey's HSD: P>0.82). *Bet* and *Leu* tended to have an increased activity in spring within krenal or both sediment types, respectively (ANOVA: $F_{(2,1),138}$=13.61 and 12.05, respectively, P<0.001, Tukey's HSD: P<0.01). *Xyl*, *Epep,* and *Est* were enzymes with generally higher activity in kryal systems (ANOVA: $F_{1,138}$=24.56, 95.46 and 20.71, respectively, P<0.001). *Epep* and *Est* showed seasonal fluctuations that were characterized by low activity in winter (ANOVA: $F_{(2,1),138}$=5.19 and 15.75, P<0.01, respectively, Tukey's HSD: P<0.001). *Phos* and *Nac* showed a strong seasonality mainly in the kryal sediments (ANOVA: $F_{2,138}$=24.46 and 7.94, respectively, P<0.001). A detailed discussion on the specific enzyme activities split by sediment type within each season can be found in the supplementary material.

Bacterial community composition

In summer, transplanting sediment of either origin into the non-native water source induced a shift in community structure. This was also true for the experiment performed in winter, but only for krenal sediments (Table 2, Figure 2B,C). In spring, no significant shift in community structure was observed in transplanted sediment (Table 2, Figure 2D). A highly significant effect of sediment origin and interaction of sediment origin and season was apparent in the total model ($F_{1,48}$=27.04, P<0.001 and $F_{1,48}$=6.74, P<0.001). Indeed, the NMDS ordination and single term PERMANOVA revealed that communities differed mainly between the two sediment origins and was influenced by a seasonal shift that was more distinct within kryal sediments (Figure 2A, Table 2).

The complete PERMANOVA model showed an interaction of treatment, incubation site, season and sediment origin, indicating a high dependency of a treatment effect on the other factors ($F_{6,48}$=1.35, P<0.05). Accordingly, a treatment effect was mainly visible in kryal sediments during summer, where the C and NP treatment in the non-transplanted and the CNP treatment in the transplanted sediments significantly influenced community structure (Table 2, Figure 2B). Also, the C treatment was different from the CNP treatment in kryal sediments during winter whether they were transplanted or not (Table 2, Figure 2C). Another C treatment effect was visible during spring in the transplanted kryal sediments (Table 2, Figure 2D).

Multivariate dispersion showed that krenal sediment bacterial communities showed, in general, less variation and therefore more stable community composition (MHGD: $F_{(1,94)}$=87.27, P>0.001). This pattern was also visible when single seasons were analyzed for dispersion differences between sediment origins (MHGD: summer: $F_{1,30}$=39.23, P>0.001, winter: $F_{1,30}$=6.04, P>0.05, spring: $F_{1,30}$=97.80, P>0.001).

Enzymatic activity structure and dynamics

There was an effect of incubation site on enzymes in all seasons, being most pronounced in spring (Table 3, Figure 3B-C). A nutrient treatment effect on kryal sediment enzymatic activity structure was mainly apparent in winter in the C treated non-transplanted sediments, whereas this treatment induced a shift in EF in spring (Table 3). Krenal sediments were more often affected by treatments (Table 3). There was an effect of CNP in summer and winter in the transplanted sediment on enzyme activity structure compared to the non-treated sediment. The non-transplanted krenal sediments were influenced by CNP treatment in winter and spring in the non-native water source (Table 3).

The complete and single PERMANOVA models revealed highly significant differences in enzymatic activity structure between the two sediment origins and a seasonal shift in sediment functioning (Table 3, Appendix Table 3 and see Figure 3A). Thus, the main patterns observed for EF are general differences between the two sediment sources with different sets of enzymes dominating the sediment types during each season.

Epep and *Est* are the primary enzymes characterizing kryal sediments (Figure 2A, Figure 3A and Appendix Figures 4d,g and 8 d, g). This pattern endures for *Epep* within the different seasons as can be seen by the vector fitting in Figure 2B-D (P<0.01). *Alph*, *Bet* and *Leu* showed mainly an activity gradient directing towards krenal sediments (Figure 2A and 3A, Appendix Figures 4 and 8). Seasonal differentiation between the two sediment types was mostly apparent during spring where krenal sediments dominated 6 enzymes, whereas *Epep and Est* still characterized kryal sediments (Figure 3D, Appendix Figure 3 and Figure 7). Enzyme activity-based NMDS ordination revealed a general seasonal shift in EF patterns. For instance, *Bet* and *Leu* showed more pronounced activity in krenal sediments in spring, whereas during winter *Phos* dominated EF in both sediment types and *Xyl* in kryal sediments (Figure 3A, Appendix Figure 8). For an additional discussion covering each season and figures of fitted GAMs of distinct enzymatic activities on ARISA NMDS and EF NMDS, consult the supplementary material.

Although enzymatic activity structure was less variable in krenal sediments when all samples were analyzed (MHGD: $F_{1,142}$=11.83, P>0.001), no difference in functional variability could be detected within each season between kryal and krenal sediment (MHGD $_{(summer, winter, spring)}$: $F_{1,46}$=2.11, 1.14, 0.88 P=0.154, 0.291 and 0.352, respectively)

Linkage of bacterial structure and function

Although a BCC shift was generally less pronounced within krenal sediments (Figure 2B-C), there was a clear change in EF in both sediment types when transplanted, indicating a response to a change in water source (Figure 3A-D). Transplanted sediments seemed to become functionally adapted to the new environment, which is apparent in the relative movements of the dispersion ellipses towards the native sediment EF activity patterns. Generally, we observed a linkage between community- and functional structure when NMDS ordinations (Figure 2A and Figure 3A) based on ARISA and enzyme activity, respectively, are compared by a procrustes test (r=0.593, P<0.001). Correlation was more strongly pronounced for kryal sediments compared to krenal sediments (r=0.588, P<0.001 and r=0.227, P=0.570, respectively) when the different sediment origins are analyzed separately.

Discussion

Our results suggest that hierarchically-structured drivers influence the patterns of bacterial community structure and functioning within hyporheic sediments in alpine running waters. Sediment origin was the main factor of influence followed by season, water source, and lastly by nutrient state.

Sediment origin: A strong delineator of BCC and EF

NMDS ordinations and the results of the complete and single-term PERMANOVAs models showed that there was a clear separation of BCC between the two sediment origins independently of any other factor (Figure 1A). This differentiation between kryal and krenal channels has been described before although across fewer sites (Logue *et al.,* 2004). Higher cell densities within krenal sediments supported this separation of BCC in the different sediment origins. This suggests that microbial communities in the two sediment types are adapted to the specific physico-chemical habitat template provided by each sediment. Spatial variability across habitats has been shown previously to be a major separator of BCC (Fortunato *et al.,* 2012). This variation can be driven by different mechanisms or environmental factors such as pH (Fierer *et al.,* 2007a), temperature (Adams *et al.,* 2010) and hydrogeochemical conditions (Battin *et al.,* 2004, Kobayashi *et al.,* 2009). Disturbance, biotic interactions and spatial connectivity also can lead to shifts in BCC (Corno *et al.,* 2008, Frey *et al.,* 2009, Langenheder *et al.,* 2011).

Bacterial function showed the same trends as those in community structure; i.e. differentiation between the two sediment origins as reported also from different systems (i.e. Romaní 2000). Distinct enzyme activities could be assigned to each sediment origin. High *Epep* and *Est* activity typically characterized kryal sediments, whereas *Bet* and *Leu* were more active in krenal sediments. This result indicates the relative importance of gathering nitrogen and carbon from distinct resources. For example, due to the relatively higher input of cellulose into krenal water systems, C may be a non-limiting resource, and thus *Bet* plays a more important role in krenal sediments (Zah and Uehlinger 2001). In kryal systems, it is probably more favorable to gather C from small ester-containing molecules like lipids and, e.g., N from peptides. Therefore, investment in *Est* and *Epep* is favored in kryal systems (Grzesiak *et al.,* 2009).

Different season: Different communities and associated functions

Seasonal change is a common observation within riverine bacterioplankton and hyporheic bacterial communities (Feris *et al.,* 2003, Crump and Hobbie 2005, Lyautey *et al.,* 2005, Olapade and Leff 2005, Crump *et al.,* 2009, Lowell *et al.,* 2009). In this study, seasonal shifts in BCC and EF were also apparent for both sediment types (Figure 2A, 3A). The significantly-fitted environmental factors on the functional NMDS indicate that the physico-chemical habitat template changed with season (Figure 3A). Seasonality in aquatic bacterial biofilm community structure and activity has been connected to the shifting physico-chemical habitat templates, and thus may also be a main driver in our study system (Battin 2000, Sekar *et al.,* 2002, Tsuchiya *et al.,* 2009, Berggren *et al.,* 2010).

Our results further showed that krenal sediments have a less pronounced seasonal BCC dynamic compared to kryal sediments, and therefore likely represent a more temporally persistent bacterial community (Figure 2A). This pattern in strength of compositional seasonality was observed within the same floodplain for a longitudinal and lateral sampling program and seems to represent a general trend in alpine floodplains (Freimann *et al.,* under review-b). The lack of strong seasonal variation in krenal BCC in their native stream habitat may be due to (I) relatively small temporal shifts in the physico-chemical habitat template within krenal systems allowing for a stable bacterial community, and/or (II) a strong potential of native krenal bacterial communities to cope with environmental changes. The latter would include physiological plasticity of krenal bacteria within the genetic level resolved in this study. Although krenal water systems are more stable concerning physico-chemical characteristics (Ward 1994, Battin *et al.,* 2004), the latter hypothesis is supported by the fact that transplanted krenal BCC shifted little but still showed strong changes in EF.

Environmental milieu and life strategies: How bacteria handle altered water source and nutritional state

Distinctions between the BCC of the two sediment origins remained clear following transplantation in all seasons. This relatively high resistance of bacterial community structure to a changing environment (here water source) has been observed in several other studies. For instance, Zumsteg *et al.* (2011) transplanted soils from glacier forefields to soils of different temperature regimes and found no assimilation of non-native communities to the new environment after 8 weeks of incubation, and only slight adaptation after 16 weeks of incubation. Waldrop and Firestone (2006) showed a soil origin effect on bacterial community composition mediated by altered environmental conditions over a two year time scale. They observed a compositional and functional community shift of transplanted oak ecosystem soil into grassland but not vice versa. The fact that we could still see the signature of sediment origin BCC when transplanted indicates a relatively autonomous native bacterial population that undergoes changes due to auto regulation than by invasion from the “outside the mesocosm world”. We would therefore expect that seasonal changes are part of a recurring and, in itself, stable successional pattern typical for each sediment origin, while the time needed for adaptation of the community to the local water source clearly extends beyond the 21 day duration of the experiment.

In contrast to BCC, the EF in transplanted sediments showed a response to a new water source, which was most pronounced for the spring season (Figure 3D). During this period of snow ablation, bacteria that show strong functional plasticity could be favored in krenal systems due diurnal fluctuations in physico-chemical habitat characteristics. A previous study showed a relative homogeneous and thus spatially weak structured BCC within the water-logged riparian zone of the krenal incubation site. Nevertheless, BCC showed a distinct shift in EF along the hydrological flow path, thus indicating a large functional plasticity by these communities during this period (Freimann *et al.,* in preparation). Although the kryal BCC did not show a significant shift after transplantation to the krenal channel during spring (Table 2), there still was a higher diversity as seen by a higher dispersion along the second axis of the NMDS (Figure 2D). This observation indicates that EF was less plastic within kryal bacterial communities.

We observed varying strengths in the coupling of structure and function in the procrustes analysis. There was a weak linkage in krenal communities between function and community structure, whereas shifts in the kryal bacterial community composition were more congruent with shifts in ecosystem function. While krenal microbes responded to transplanting by changing their functional output without fundamentally changing their BCC, kryal bacteria changed their EF by restructuring the community to one able to cope with the new water source. This suggests that different sediment origins may have a different level of functional redundancy and plasticity (Allison and Martiny 2008).

It has been shown that the linkage of species composition to ecosystem function is not written in stone but rather seems to be a continuum from strong functional linkages to functional redundancy and plasticity depending on the complexity of the investigated system and the scope in functions (Fernandez *et al.,* 2000, Kirchman *et al.,* 2004, Langenheder *et al.,* 2006, Boucher and Debroas 2009, Comte and Del Giorgio 2010, Brankatschk *et al.,* 2011, Frossard *et al.,* 2012). Nevertheless, in the context of our direct comparison, krenal sediments appear to be habitats for bacterial generalists that do not reorganize their community structure to environmental changes, at least within the magnitude of change and temporal scope covered in this study. In contrast, kryal sediment bacteria showed a simultaneous transition in community structure and EF, suggesting kryal bacterial communities act more as functional specialists. The fitting of bacteria taxonomic units (OTU’s) on the EF NMDS in all three experiments revealed a high proportion of OTU’s that were observed primarily in krenal sediments (Figure 3A). The strong relationship of these OTU’s with krenal sediments is further reflected in their high abundance throughout the year. They perform well as generalists within krenal sediments but are not able to colonize kryal BCC within the duration of the experiment when transplanted.

Olapade and Leff (2005, 2006) examined the influence of DOM and inorganic nutrients on stream biofilm bacterial community and found a significant positive treatment effect on total bacterial abundance and shift in BCC mainly induced by labile DOM. They also found seasonal dependency on how BCC and abundance changed. Crump *et al.* (2003) correlated the quality of organic matter with seasonal changes in BCC and productivity in arctic lakes. Other studies conducted with planktonic and heterotrophic biofilm BCC revealed the same trends we found in which biomass accumulation is minor compared to, e.g., changes in functional processes and productivity with amended nutrient levels (Jansson *et al.,* 2006, Van Horn *et al.,* 2011).

The relative lack of response in bacterial abundance to nutrient status in this study could be due to various mechanisms. Alpine streams, in general, have low amounts of DOC and lower temperatures compared to streams in the studies above. The BCC

in alpine systems may lack the functional ability to incorporate additional readily-available C into biomass, at least within the 3 week experimental period in our study. However, a combined effect of water source and nutrient treatment on bacterial abundance was found in winter. A negative feedback of added nutrients on krenal sediment bacterial abundance when placed in a new environment contrasted with the positive feedback of transplanted kryal sediments. The contrasting influence of N+P and the combined treatment with C on bacterial abundances during winter indicates a complex interaction with water source and native bacterial communities between seasons rather than a general, growth-inducing effect by nutrients. Another aspect may be differences in the general experimental set-ups and the used resources and their applied concentrations in the different studies. For instance, Olapade and Leff (2005, 2006) used clay pots filled with relatively high concentrations of a glucose solution (~1 mol l^{-1}) as artificial substratum in one experiment, thus instilling DOC directly into the biofilm.

The native kryal bacterial communities showed highest diversity within each season. This pattern indicates that diversity in kryal sediments is increased by transplantation and nutrient treatment effects. Although the PERMANOVA results do not reveal a generally significant shift in community composition due to nutrient addition, kryal sediment BCC were more influenced by the amended nutrient state (see Table 2). The more extended dispersion ellipses of kryal sediments in the NMDS plots (Figure 2B-D) further support this finding. The relative weak response pattern of BCC to nutrient amendment could be due to the relative low increase in nutrient concentrations. For example, Bowen et al. (2009) found that even a severe increase (15x above background) in N and P in salt marsh sediments does not necessary lead to a shift in BCC. (Lage *et al.,* 2010) found within the same habitat a shift in ammonia-oxidizing bacteria composition when N and P were applied separately. This effect was not present when N and P were applied together. They also found temporal variation in the response to the treatments and concluded that an interactive effect with temporally variable abiotic factors and taxa specific preferences for nutrient concentrations drove the compositional variability. The relatively weak response to the nutrient treatments in our experiment may also depend on interactive effects. As kryal communities may harbor taxa that have narrow preferences in nutrient concentrations (i.e., specialists), there would be consequently a larger shift in kryal BCC.

Conclusions on microbial transformations in alpine floodplains

BCC shifts appeared to be dependent on bacterial functional abilities driven by changing environmental constraints. When sediments were transplanted or amended with nutrients, there was a persistence effect related to sediment origin dictating the outcome. For example, resistance of BCC after transplantation was, according to statistical analysis, linked to the functional abilities of single OTU's. This fact suggests that distinct strategies of different bacteria contribute to the assembly and functional maintenance of bacterial communities. Global change induced changes in water sources in glacial forefields would therefore be expected to go along with widespread shifts in microbial communities, at least in the long term. A future loss in bacterial diversity could be expected when resident kryal specialists are unable to compete within an environment dominated and invaded by krenal OTU's. It has been shown that biodiversity and environmental complexity are interactively linked to ecosystem functioning, thus an effect on water properties is possible when ecosystem diversity and homogeneity are reduced (Wagner *et al.,* 2002, Langenheder *et al.,* 2010). As transplanted kryal BCC was not substantially more similar to krenal BCC as non-transplanted sediments during our experimental span, it is not apparent if a similar BCC as in krenal sediments will develop. Regardless, long-term studies incorporating colonization dynamics would be needed to assess if kryal BCC could persist or if there would be complete convergence of kryal BCC towards a krenal assemblage.

Interestingly, there was considerable functional flexibility in both sediment types independent of any underlying mechanism. Thus a shift from kryal to krenal water dominated systems may be buffered by a lag in the functional response by microbes. Although microbial functionality does not collapse and adapt to a new environment, such a shift will still have implications on carbon and nitrogen cycles at the ecosystem scale (Shen and He 2011). For instance, decomposition rates were shown to be influenced by historic imprints on BCC (Strickland *et al.,* 2009). EF was reduced when BCC faced an unfamiliar habitat. It is possible that future change in EF will be slowed down at some time point and that similar EF emerges at a temporal scale relevant for shaping sediment conditions in streams. Predictions concerning future shifts in EF and their impact on biogeochemical cycling should consider apparent BCC and functional flexibility, as they guide possible future shifts in alpine ecosystems facing novel environmental conditions.

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Titles and legends to figures

Figure 1

Experimental set-up. Arrows depict the incubation sites water source whereas cylinders illustrate the mesocosms. Treatments and the sediment origin are annotated within the cylinders.

Figure 2

NMDS of ARISA profiles. Dots depict ordination of bacterial community structure. Annotation is as follows: first letter describes sediment origin (g = kryal, r = krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP = nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g (**g**lacial) = kryal, r = k**r**enal) and last capital letter names the season the experiment was conducted (A = summer, O = winter, M = spring). Light and dark blue color of the dots corresponds to the distinct sediment origins and the size is relatively to the numbers of OTU's. Strength and direction of fitted enzyme activity gradients are depicted as arrows. Orange arrows are enzymes fitted with permutational power of $P<0.05$ and blue ones with $P<0.1$ (See methods for abbreviations). Ordinations are split in panel (A) for all seasons, (B) for summer, (C) for winter and (D) spring. Dispersion of standard error of the weighted scores of factors (Seasons in panel A and sediment origin x incubation site in panels B to C) are depicted as ellipses.

Figure 3

NMDS of enzymatic activities. Dots depict samples in ordination based on enzymatic activity structure. Annotation is as follows: first letter describes sediment origin (g = kryal, r = krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP= nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g = kryal, r= krenal) and last capital letter names the season the experiment was conducted (A (August) = summer, O (October) = winter, M (May) = spring). Colors of the dots correspond to the distinct sediment origins and the size is relatively to the log of the sum of the measured enzymes activities. Strength and direction of fitted water chemistry gradients are depicted as arrows. The blue arrows are chemical characteristics which fitted with permutational power of $P<0.05$. Ordinations are split in panel (A) for all seasons, (B) for summer, (C) for winter and (D) spring. Dispersion of standard error of the weighted scores of factors (Sediment origin x Seasons in panel A and sediment origin x incubation site in panels B to D) are depicted as ellipses. Grey arrows depict fitted OTU's. Light grey arrows were fitted with $P<0.001$ and dark grey ones with $P<0.0001$ in panel A and D, with $P<0.01$ and 0.001 in panel B and C.

Figure 1

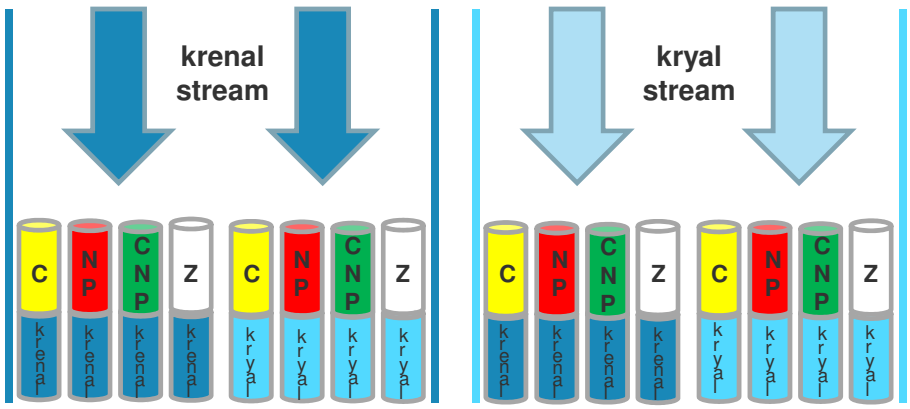


Figure 2

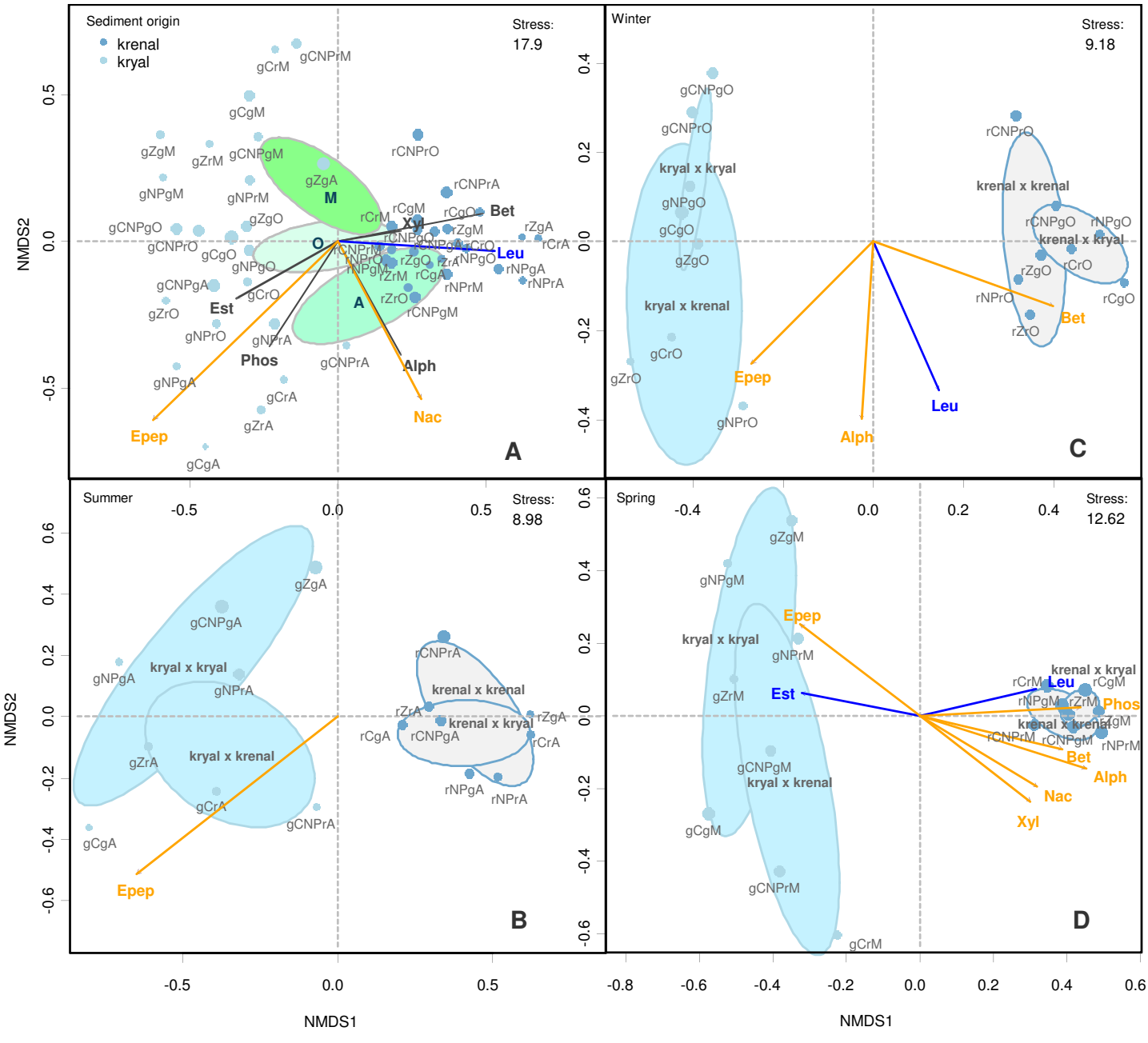


Figure 3

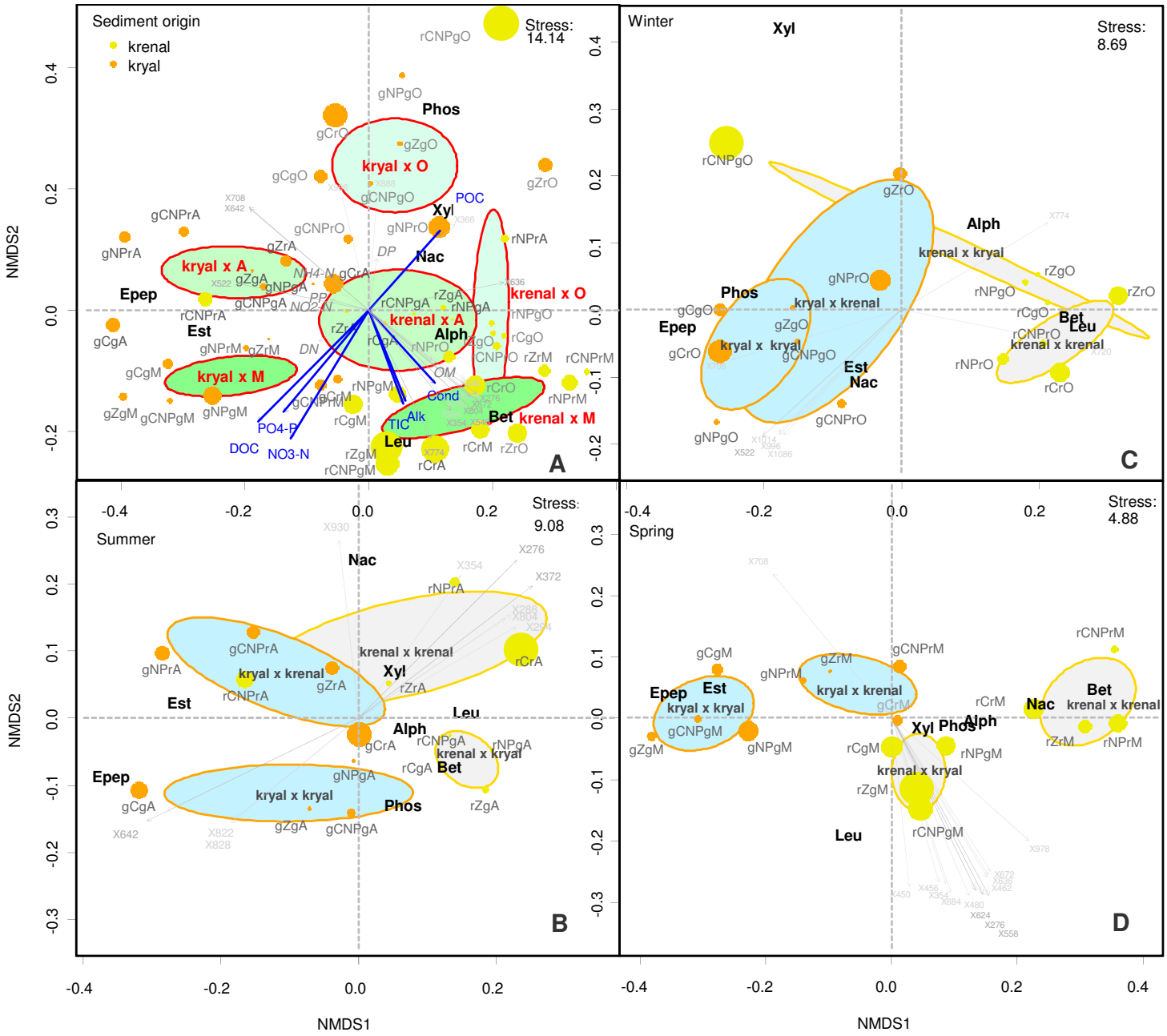

Sediment origin
krenal
kryal
Stress: 14.14
A
Winter
Stress: 8.69
C
Summer
Stress: 9.08
B
Spring
Stress: 4.88
D
NMDS1
NMDS2
kryal x A
kryal x O
kryal x M
krenal x A
krenal x O
krenal x M
kryal x krenal
krenal x krenal
kryal x kryal
krenal x kryal
Epep
Est
Nac
Phos
Alph
Bet
Leu
Xyl
DP
PP
DN
OM
Cond
Alk
TIC
DOC
NO3-N
PO4-P
NH4-N
NO2-N
POC

Table 1 Repeated measures ANOVA for bacterial abundance and enzymatic activities. Treatment (T), Incubation Site (IS) and Sediment Origin (SO) were used as independent variables, Season (S) is the repeated variable.

Chi^2-statistic Parameter	Treatment (T)	Incubation site (IS)	Season (S)	Sediment origin (SO)	T x IS	T x S	IS x S	T x SO	IS x SO	S X SO	T x IS x S	T x IS x SO	T x S x SO	IS x S x SO	T x IS x S x SO
dF	3	1	2	1	3	6	2	3	1	2	6	3	6	2	6
DAPI	3.94	0.08	19.12***	11.76***	5.17	18.07**	1.53	2.73	2.15	18.96***	25.6***	5.92	49.95***	0.47	12.48
Number of OTUs	5.35	0.04	5.39	0.05	2.13	11.38	1.53	9.14*	0.68	6.11*	10.51	15.71**	28.29***	0.57	31.28***
Total Enzyme	5.16	9.89**	0.07	0.00	6.16	5.76	5.09	1.42	2.95	1.84	6.17	6.52	12.09	1.38	13.02*
α-Glucosidase	4.21	0.05	2.86	0.04	4.15	6.81	0.63	4.51	0.96	7.56*	5.3	5.26	15.86*	0.9	13.14*
β-Glucosidase	1.52	0.40	21.38***	1.39	2.17	3.46	1.96	3.38	5.84*	30.58***	5.67	5.67	18.46**	8.27*	17.39**
Xylosidase	0.87	0.70	0.13	1.15	1.32	4.42	1.13	0.28	0.06	0.83	3.15	0.85	5.53	0.12	5.54
N-acetyl-glucasaminidase	8.14*	1.51	9.25**	1.97	4.10	9.04	1.56	3.22	3.76	25.19***	3.97	2.75	16.05*	5.68	8.22
Esterase	10.04*	4.22*	2.81	2.15	5.06	14.44*	12.64*	5.00	0.59	2.47	6.49	7.72	13.09*	0.77	11.99
Leucine-aminopeptidase	15.33**	0.36	14.61**	1.71	14.07**	14.77*	17.30***	6.35	3.66	19.18***	15.39*	10.83*	30.62***	4.73	28.23***
Endopeptidase	1.18	6.06*	20.42***	15.38***	0.88	1.60	14.71***	0.83	0.08	10.74**	7.28	1.86	13.46*	6.44*	4.95
Phosphatase	4.26	1.47	4.47	1.33	6.58	5.78	3.80	0.66	2.40	27.45***	5.64	3.63	14.79*	6.77*	15.71*

*** P<0.001

** P<0.01

* P<0.05

Table 2 PERMANOVAs of ARISA profiles (n=2). Structured pairwise comparison for Sediment Origin (df(1,95)), Season (df(2,47)), Incubation Site (df(1,22)), and Treatment (df(1,7)). Dark grey shading indicates the kryal incubation site, light grey indicates the krenal incubation site.

Sediment Origin

18.95***

				kryal							krenal			
Season	Season	Incubation site	Treatment	Z	C	NP	CNP	Season	Incubation site	Treatment	Z	C	NP	CNP
Summer		1.66*	Z		2.24**	1.65*	0.92		1.96*	Z		0.76	1.98*	1.47
			C	0.90		1.64*	1.20			C	1.23		1.45	0.87
			NP	1.23	0.92		1.06			NP	1.43	0.96		0.88
			CNP	2.23**	0.99	1.26				CNP	0.99	0.84	1.29	
Winter		1.52	Z		1.17	1.29	1.25		2.29*	Z		1.31	0.63	0.51
			C	0.98		1.33	2.15**			C	1.1		1.98	0.66
	5.21***		NP	1.13	1.59		1.44	6.73***		NP	0.31	0.71		1.07
			CNP	1.50	2.04*	1.69				CNP	2.12*	1.89	1.51	
Spring		1.04	Z		1.54	0.98	1.44		1.11	Z		0.82	1.4	2.49*
			C	1.78*		1.92*	1.18			C	0.89		1.12	2.02
			NP	0.49	1.45		0.96			NP	1.55	1.25		0.39
			CNP	1.14	0.85	1.50				CNP	0.98	0.85	1.29	

incubation site:
kryal
krenal
*** P<0.001
** P<0.01
* P<0.05

Table 3 PERMANOVAs of enzymatic activity profiles (n=3). Structured pairwise comparison for Sediment Origin (df(1,143)), Season (df(2,71)), Incubation Site (df(1,23)), and Treatment (df(1,11)). Dark grey shading indicates the kryal incubation site, light grey indicates the krenal incubation site.

Sediment Origin

53.05***

kryal								krenal							
Season	Season	Incubation site	Treatment	Z	C	NP	CNP	Season	Incubation site	Treatment	Z	C	NP	CNP
Summer	21.61***	3.48**	Z		1.36	0.33	0.40	9.98***	9.46***	Z		2.04	1.28	3.24**
			C	0.62		1.59	1.50			C	1.11		1.82	1.31
			NP	1.32	0.88		0.27			NP	1.24	1.52		0.88
			CNP	1.04	0.61	0.18				CNP	0.42	1.3	2.03	
Winter		2.89*	Z		1.45	2.40	1.04		6.33***	Z		0.28	0.19	4.00**
			C	1.63		3.81*	1.05			C	0.75		0.11	3.66**
			NP	0.02	1.36		2.48			NP	1.02	0.06		3.06*
			CNP	0.97	1.38	0.55				CNP	3.54**	2.61*	2.64*	
Spring		15.94***	Z		2.48	1.57	0.70		47.65***	Z		1.51	0.94	0.33
			C	3.31*		0.77	0.72			C	2.12		1.42	3.41*
			NP	0.09	2.20		0.38			NP	0.24	2.88*		2.1
			CNP	0.89	1.85	0.86				CNP	3.63**	4.21**	2.92*	

incubation site

kryal

krenal

*** P<0.001

** P<0.01

* P<0.05

Supplementary information

Additional results of bacterial abundance

The influence of treatment was mainly visible during the winter experiment in the transplanted sediments; The native kryal sediment showed an interaction with treatment and incubation site (ANOVA: $F_{(3,16)}$=34.85, P<0.001). A post-hoc test revealed increased and distinct cell numbers due to all treatments within the transplanted kryal sediments (Tukey's HSD: P<0.05). The native krenal sediment also showed an interaction with treatment and incubation site during winter (ANOVA: $F_{(3,16)}$=71.34, P<0.001). Here, a post-hoc test showed a significant reversed trend with decreased cell numbers in the transplanted sediments due to the different treatments and an increased cell numbers within the non-transplanted CNP treated sediments (Tukey's HSD: P<0.05). A comparably weaker effect of treatment was also apparent in spring in kryal and krenal sediments (ANOVA: $F_{(3,16)}$=5.09 and 8,15 P<0.05 and P<0.01 respectively.). The kryal sediments showed the same pattern for a distinct treatment but with generally higher cell abundance within the transplanted sediments (ANOVA: $F_{(1,16)}$=19.82, P<0.001).

Consult supplementary information Table 1 for in-detail abundance differences.

Additional results on enzymatic activities

Total enzyme activity (sum of the measured enzymes) was significantly affected by treatment, incubation site, season and sediment origin (ANOVA: $F_{(6,96)}$=6.06, P<0.05).

Alph showed decreased activity in transplanted krenal sediment in summer (ANOVA: $F_{(1,16)}$=17.72, P<0.001). A NP and CNP treatment effect on Alph was observed during spring in kryal sediments, which showed increased activities due to the NP and CNP amendment compared to the untreated (zero treatment) mesocosms (ANOVA: $F_{(3,16)}$=4.01, P<0.05, Tukey's HSD: P<0.05).

Bet showed a decreased activity in transplanted krenal sediments in spring (ANOVA: $F_{(1,16)}$=55.08, P<0.001). The transplanted kryal sediment had an increased *Bet* activity in winter (ANOVA: $F_{(1,16)}$=13.34, P<0.001). *Bet* was affected by the CNP treatment in kryal sediments in spring, which showed higher activity compared to the untreated mesocosms (ANOVA: $F_{(3,16)}$=3.71, P<0.05, Tukey's HSD: P<0.05).

Transplanted krenal sediments had decreased *Xyl* activity in summer (ANOVA: $F_{(3,16)}$=38.24, P<0.001). *Xyl* of krenal sediments were influenced by treatment and incubation site in spring (ANOVA: $F_{(3,16)}$=6.37, P<0.01 and $F_{(1,16)}$=14.48, P<0.01 respectively). A post-hoc test showed that the CNP treatment significantly decreased *Xyl* activity in krenal sediments in spring (Tukey's HSD: P<0.05). *Xyl* activity was increased after transplanting kryal sediments to the non-native water source during summer, winter and spring (ANOVA: $F_{(1,16)}$=5.20, 8.26 and 5.83 respectively, P<0.05).

Krenal sediment *Est* activity showed an incubation site effect with higher values in summer and winter when kept in the native water source (ANOVA: $F_{(1,16)}$=15.25, 5.44, P<0.01 and P<0.05, respectively). A treatment and incubation site effect on *Est* activity was visible in spring in krenal sediments (ANOVA: $F_{(1,16)}$=8.45, 75.67, P<0.01 and P<0.001, respectively). In contrast to the other seasons, there was an increase in Est activity after transplant and a lower *Est* activity of CNP treated compared to C treated sediments (Tukey's HSD: P<0.001). Kryal sediments showed an incubation site effect in summer with generally highest *Est* activity in the non-native water source (ANOVA: $F_{(1,16)}$=10.93, P<0.01).

Krenal sediments showed an incubation site effect on *Epep* with higher activities during summer in the native water source, whereas activities were increased in winter and spring for transplanted krenal sediments (ANOVA: $F_{(1,16)}$=9.33, 6.28, 45.61 , P<0.01, P<0.05 and P<0.001). There was a treatment effect during spring on krenal sediments where CNP treatment showed decreased *Epep* activity compared to C treated sediments (ANOVA: $F_{(3,16)}$=5.04, P<0.05, Tukey's HSD: P<0.01). Kryal sediment *Epep* activity was influenced by the C treatment during winter and showed higher activity compared to the zero and CNP treatment (ANOVA: $F_{(3,16)}$=4.83, P<0.05, Tukey's HSD: P<0.05). Transplanted kryal sediment had generally lower *Epep* activity during the spring experiment (ANOVA: $F_{(1,16)}$=15.23, P<0.01).

Nac activity was decreased during summer and spring for transplanted krenal sediment (ANOVA: $F_{(1,16)}$=22.18, 8.67, P<0.001 and P<0.01, respectively). There was an additional effect of CNP during spring, which decreased *Nac* activity compared to the NP treatment in krenal sediments (ANOVA: $F_{(3,16)}$=3.67, P<0.05, Tukey's HSD: P<0.05). Transplanted kryal sediment showed increased *Nac* enzyme activity in summer and spring (ANOVA: $F_{(1,16)}$=24.45, 5.58, P<0.001 and P<0.05, respectively).

Leu activity was decreased in transplanted krenal sediments in summer and winter (ANOVAs: $F_{(1,16)}$=8.03, 13.24, P<0.05 and P<0.01, respectively). An interaction of incubation site and treatment affecting *Leu* activity in spring was visible (ANOVA:$F_{(3,16)}$=10.57, P<0.001). A post-hoc test showed that non-transplanted krenal sediments were less active compared to transplanted untreated krenal sediments (Tukey's HSD: P<0.05). In addition, CNP treated non-transplanted sediment showed lower activity compared to all krenal sediments (Tukey's HSD: P<0.001). Kryal sediments showed an incubation site effect during winter with increased activities for *Leu* in transplanted sediments (ANOVA:$F_{(1,16)}$=12.66, P<0.01). There was an interaction of treatment and incubation site on *Leu* activity in kryal sediments in spring (ANOVA:$F_{(3,16)}$=3.55, P<0.05). A post-hoc test showed that transplanted kryal sediment in spring showed decreased activity compared to the transplanted C treated sediment and the zero and NP treated non-transplanted sediments (Tukey's HSD= P<0.05).

Krenal sediments *Phos* activity was influenced by incubation site and treatment during spring (ANOVAs: $F_{(1,3\text{-}16)}$= 8.44, 5.79, P<0.05 and P<0.01, respectively). Transplantation reduced *Phos* activity of krenal sediments during spring (Tukey's HSD: P<0.05). CNP treatment decreased krenal sediment *Phos* activity compared to the other treatments (Tukey's HSD: P<0.05). Kryal sediment *Phos* activity was influenced by CNP treatment, which showed lower activity compared to C treated sediments (ANOVA: $F_{(3,16)}$=3.36, P<0.05 Tukey's HSD: P<0.05). In spring, kryal sediments showed an interaction of treatment and incubation site on *Phos* activity (ANOVA: $F_{(3,16)}$=4.41, P<0.05). Kryal sediment that was transplanted showed lower Phos activity then transplanted CNP treated and non-transplanted NP treated sediments (Tukey's HSD: P<0.05).

Additional discussion on the multivariate correlations between enzyme activity and community structure

A refined picture of enzyme activities within the community structure can be seen when each single seasonal experiments community- and activity structures are ordinated.

During the summer experiment, *Epep* was mainly fitted on the community NMDS (P<0.01) with a gradient towards kryal sediments directing more to the transplanted sediments (Figure 2B, 3B, Appendix Figure 1b). Although the BCC shift was not strongly pronounced, there was a clear change in EF due to water source. Transplanted sediments seemed to become functionally adapted to the new environment (Figure 2B, Figure 3B).

During the winter experiment, *Alph* was more expressed in the krenal and transplanted kryal sediments, whereas *Bet* was mainly active in krenal sediments (vector fittings: P<0.05) (Figure 2C, Figure 3C, Appendix Figure 2A). *Epep* showed the strongest change in gradient, in the direction of kryal sediments in winter (vector fitting: P<0.05) (Figure 2C, Figure 3C, Appendix Figure 2b). Although the community structure of krenal sediments shifted only slightly in winter due to the changed water source, functioning changed within both sediment origins (Figure 2C, Tables 2 and 3).

The spring season revealed more distinct enzymatic activity between the two sediment origins. Six measured enzymes showed a good fitting on the community NMDS (P<0.05) (Figure 2D). *Epep* and *Est* were most active within kryal non-transplanted communities (Figure 2D, Figure 3D, Appendix Figure 3a,b). *Leu*, *Nac*, *Phos* and *Bet* showed a linear strength of gradient in activity towards krenal sediments (Figure 2D, Figure 3D, Appendix Figure 3b). *Xyl* and *Alph* showed peak activity values in transplanted kryal sediments and generally in krenal sediments in spring (Figure 2D, Figure 3D, Appendix Figure 3a). There was no shift in community composition due to transplanting, but a strong change in functioning during this period with functioning shifting towards the native community functions for transplanted sediments (Figure 2D, Fig 3D, Tables 2 and 3). Fitting of OTU's on the enzymatic activity ordination of all seasons revealed 17 OTU's that were fitted with permutational power P<0.001 and 9 with P<0.0001. Three OTU's were fitted with permutational power of P<0.001 in the enzymatic activity ordination in summer, 7 OTU's with P<0.01 in winter and 13 OTU's with P<0.001 in spring. The directions of gradient of significantly fitted OTU's were clearly separated between kryal and krenal originated sediments indicating diversified BCC depending strongly on these fitted OTU's within the sediment type (Figure 3).

Supplementary information figure legends

Figures 1 to 8 are based on the ordinations of the main manuscript.

Appendix Figure 1a to Figure 3b

NMDS of ARISA profiles within single seasons; 1a,b: summer; 2a,b: winter; 3a,b:spring. Dots depict ordination of bacterial community structure. Annotation is as follows: first letter describes sediment origin (g = kryal, r = krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP = nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g=kryal, r=krenal) and last capital letter names the season the experiment was conducted (A = summer, O = winter, M = spring). Light and dark blue colors of the dots correspond to the distinct sediment origins and the size is relatively to the log of the sum of the measured enzymes activities. Orange contour line show values of single enzymes as calculated by a general additive model (GAM). The percentages of explained deviance of the GAM are given. Dispersion of standard error of the weighted scores of factors (sediment origin x incubation site, confidence limit = 0.95) are depicted as ellipses.

Appendix Figure 4a to Figure 4h

NMDS of ARISA profiles of all seasons. Dots depict ordination of bacterial community structure. Annotation is as follows: first letter describes sediment origin (g = kryal, r = krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP = nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g = kryal, r = krenal) and last capital letter names the season the experiment was conducted (A = summer, O = winter, M = spring). Light and dark blue color of the dots corresponds to the distinct sediment origins and the size is relatively to the log of the sum of the measured enzymes activities. Orange contour line show values of single enzymes as calculated by a general additive model (GAM). The percentages of explained deviance of the GAM are given**.**

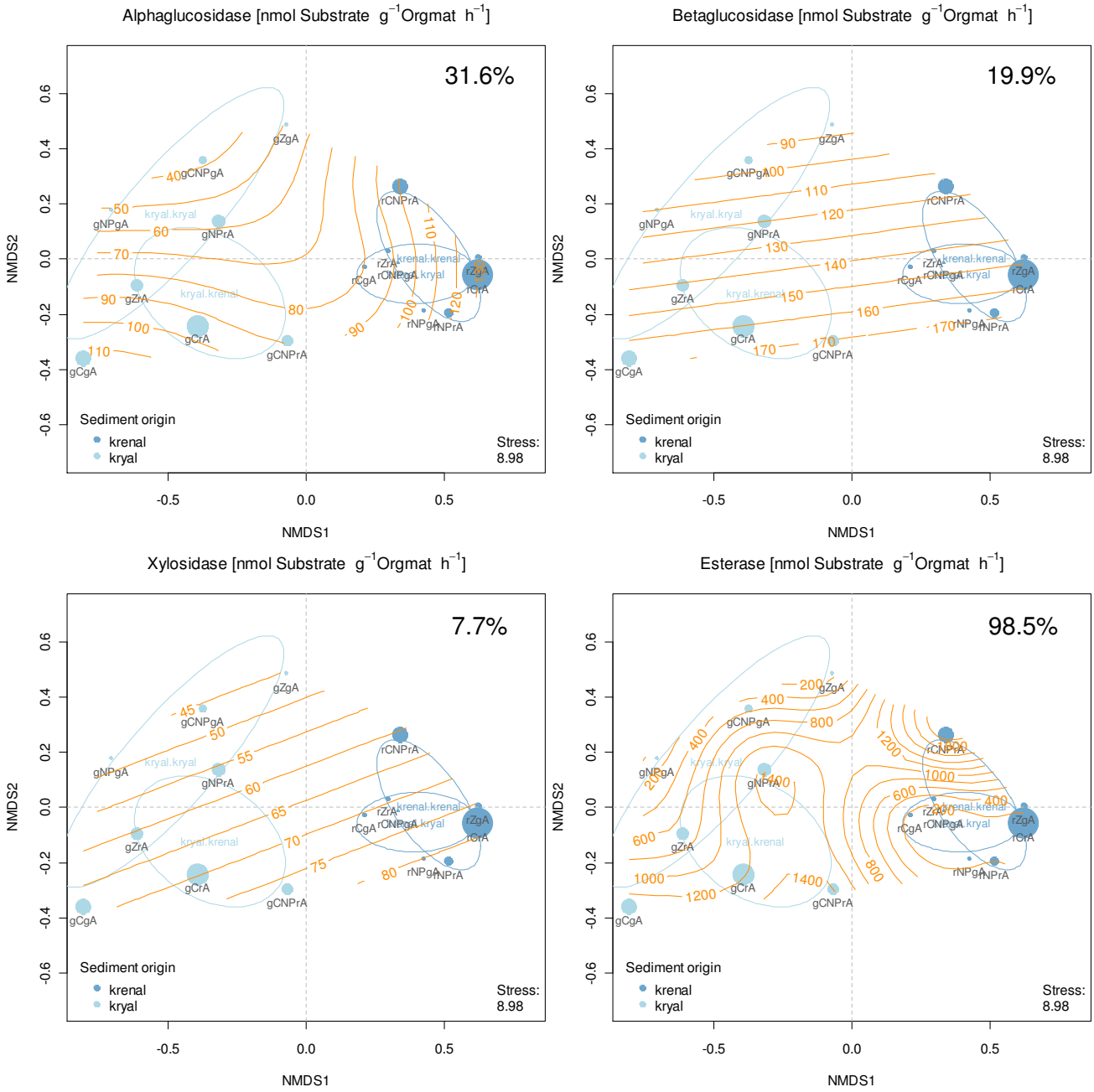
Appendix Figure 5a to Figure 7b

NMDS of enzymatic activity profiles within single seasons; 1a,b: summer; 2a,b: winter; 3a,b:spring. Dots depict ordination of bacterial community structure. Annotation is as follows: first letter describes sediment origin (g = kryal, r = krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP = nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g=kryal, r=krenal) and last capital letter names the season the experiment was conducted (A = summer, O = winter, M = spring). Yellow and orange colors of the dots correspond to the distinct sediment origins and the size is relatively to the log of the sum of the measured enzymes activities. Orange contour line show values of single enzymes as calculated by a general additive model (GAM). The percentages of explained deviance of the GAM are given.

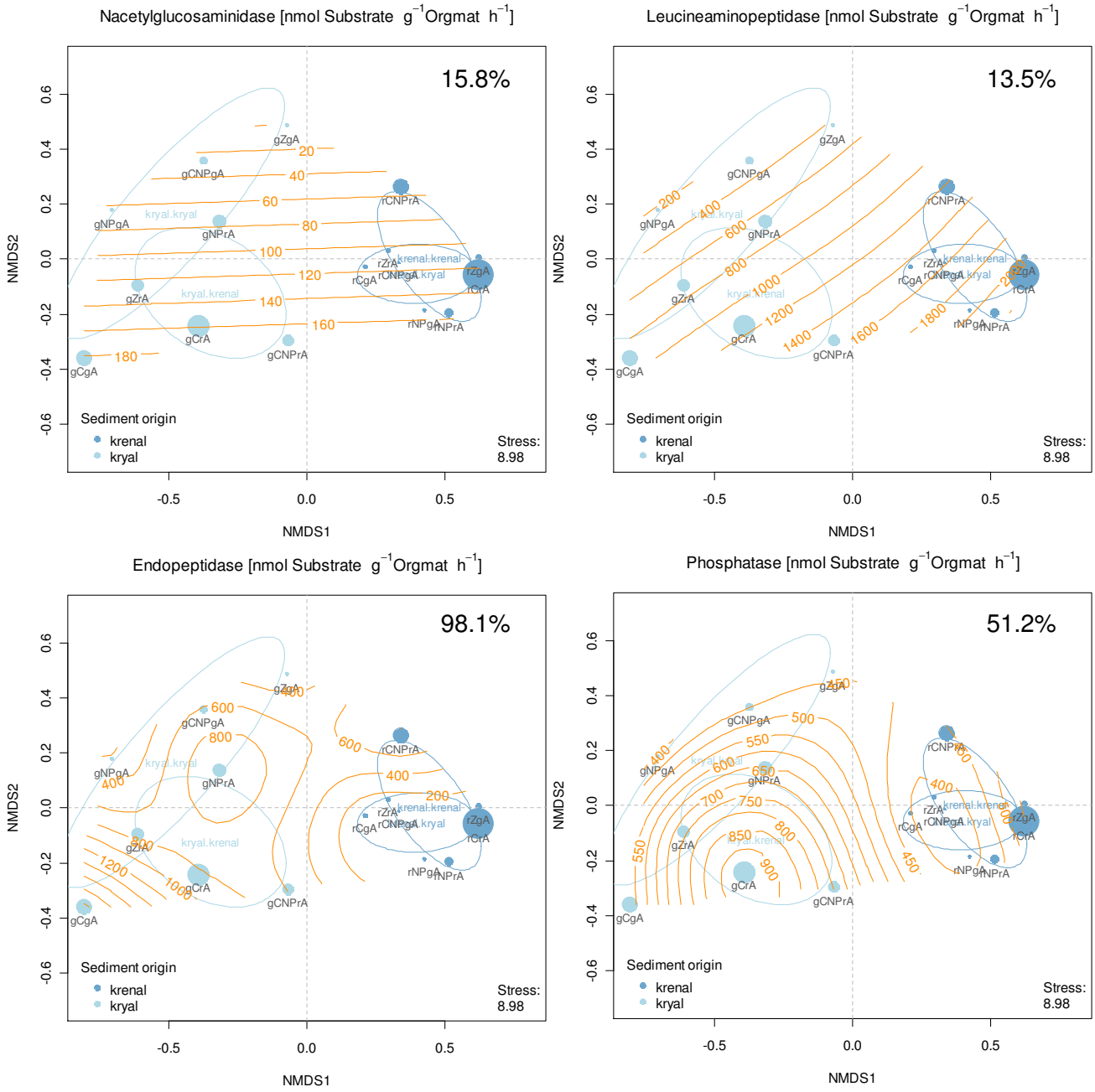
Appendix Figure 8 a to h

NMDS of Enzyme profiles of all seasons. Dots depict ordination of enzymatic community structure. Annotation is as follows: first letter describes sediment origin (g=kryal, r=krenal), capital letters in the middle describe the treatment (Z = null, C = carbon, NP = nitrogen and phosphorous, CNP = carbon, nitrogen and phosphorous), next letter describes the incubation site (g = kryal, r = krenal) and last capital letter names the season the experiment was conducted (A = summer, O = winter, M = spring). Yellow and orange color of the dots corresponds to the distinct sediment origins and the size is relatively to the log of the sum of the measured enzymes activities. Orange contour line show values of single enzymes as calculated by a general additive model (GAM). The percentages of explained deviance of the GAM are given.

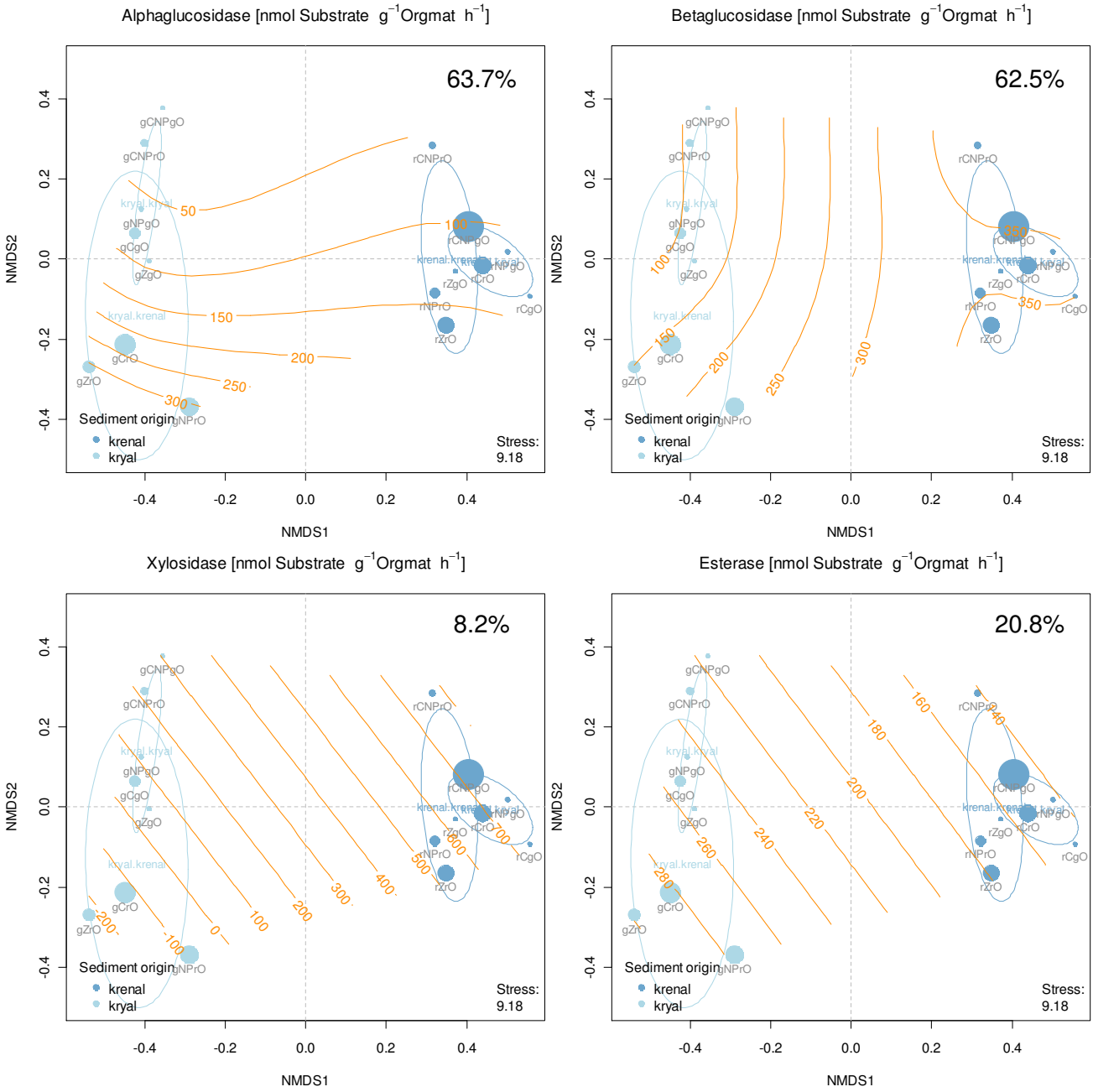
Appendix Figure 1a



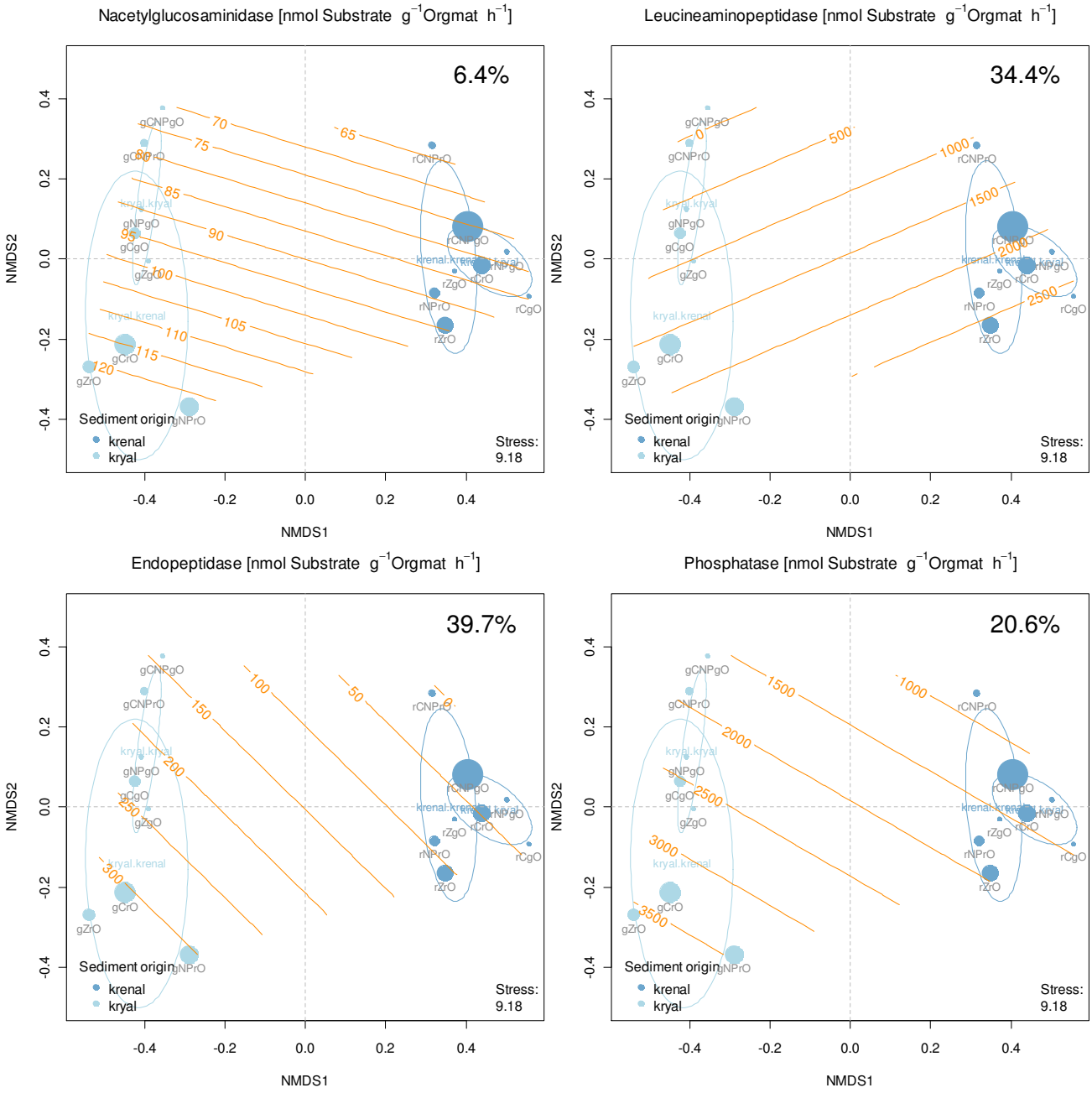
Appendix Figure 1b



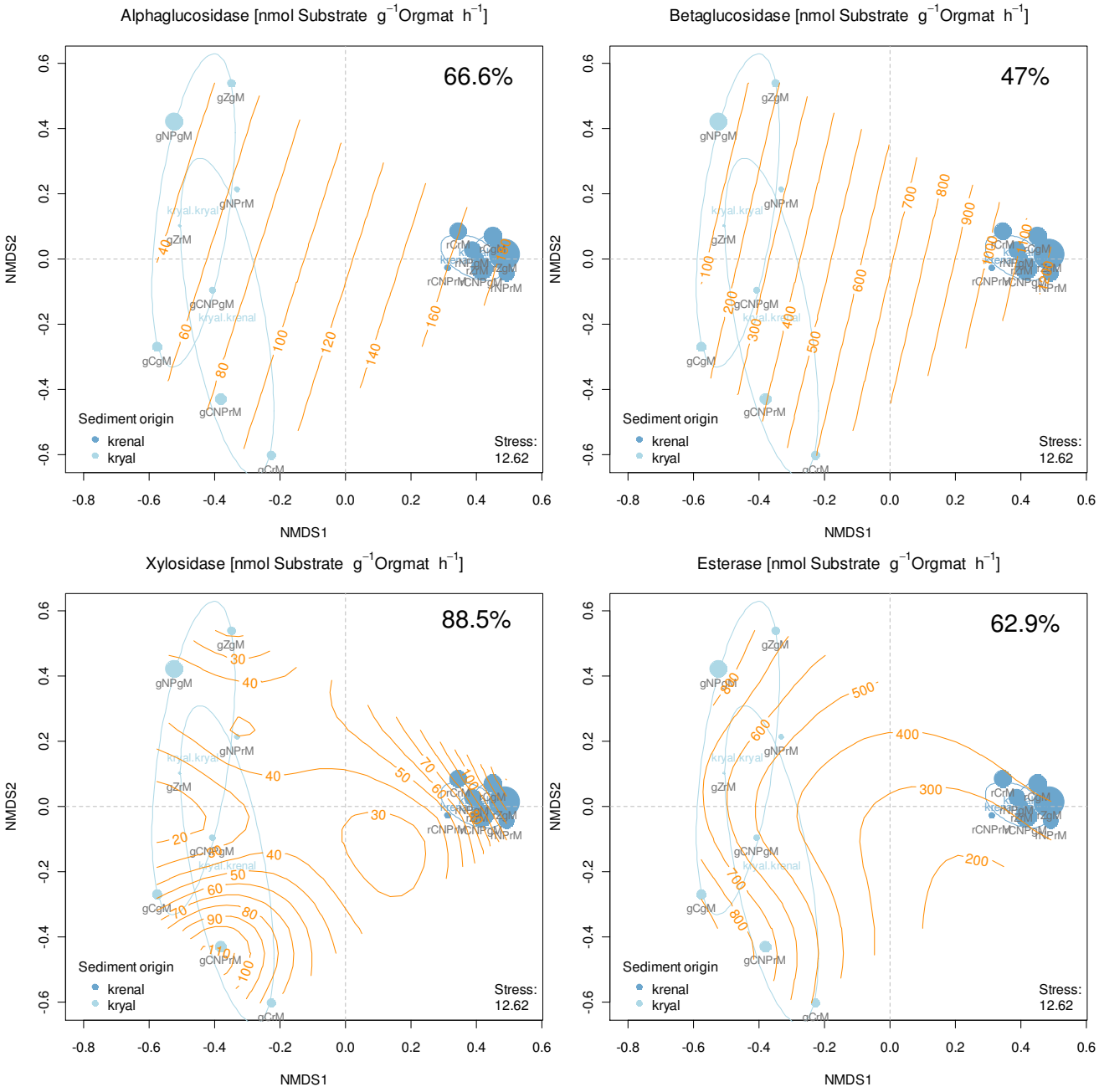
Appendix Figure 2a



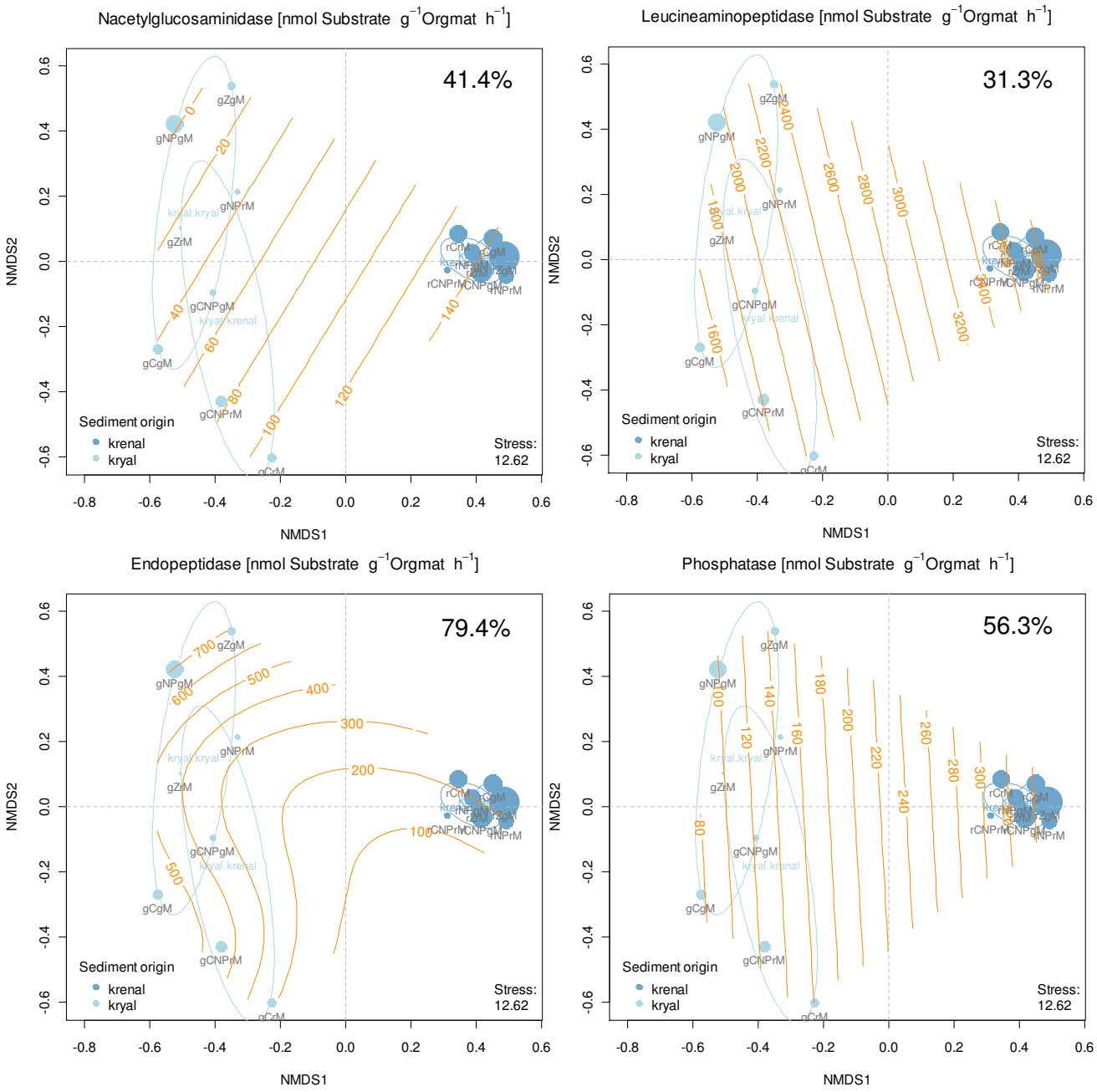
Appendix Figure 2b



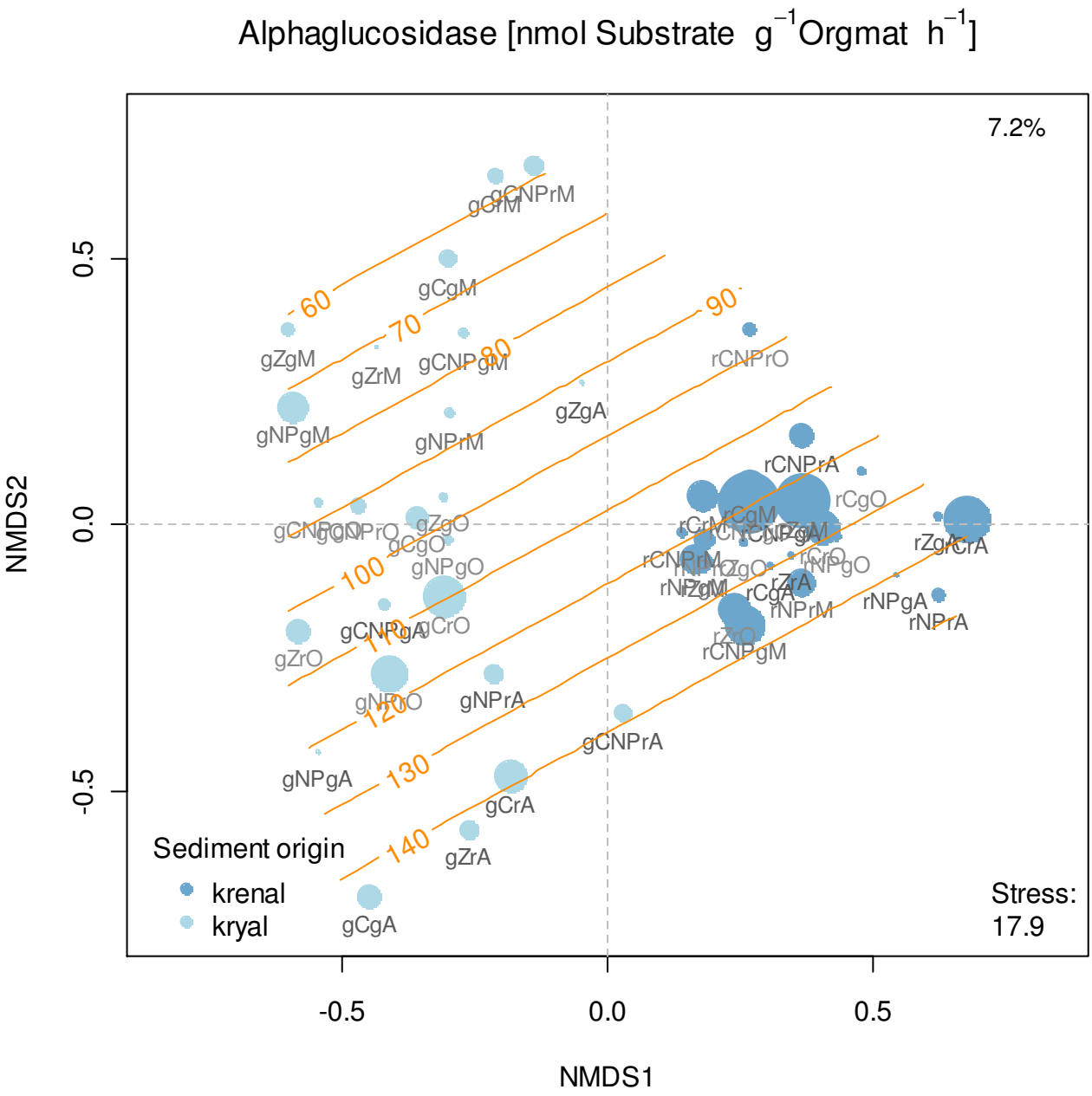
Appendix Figure 3a



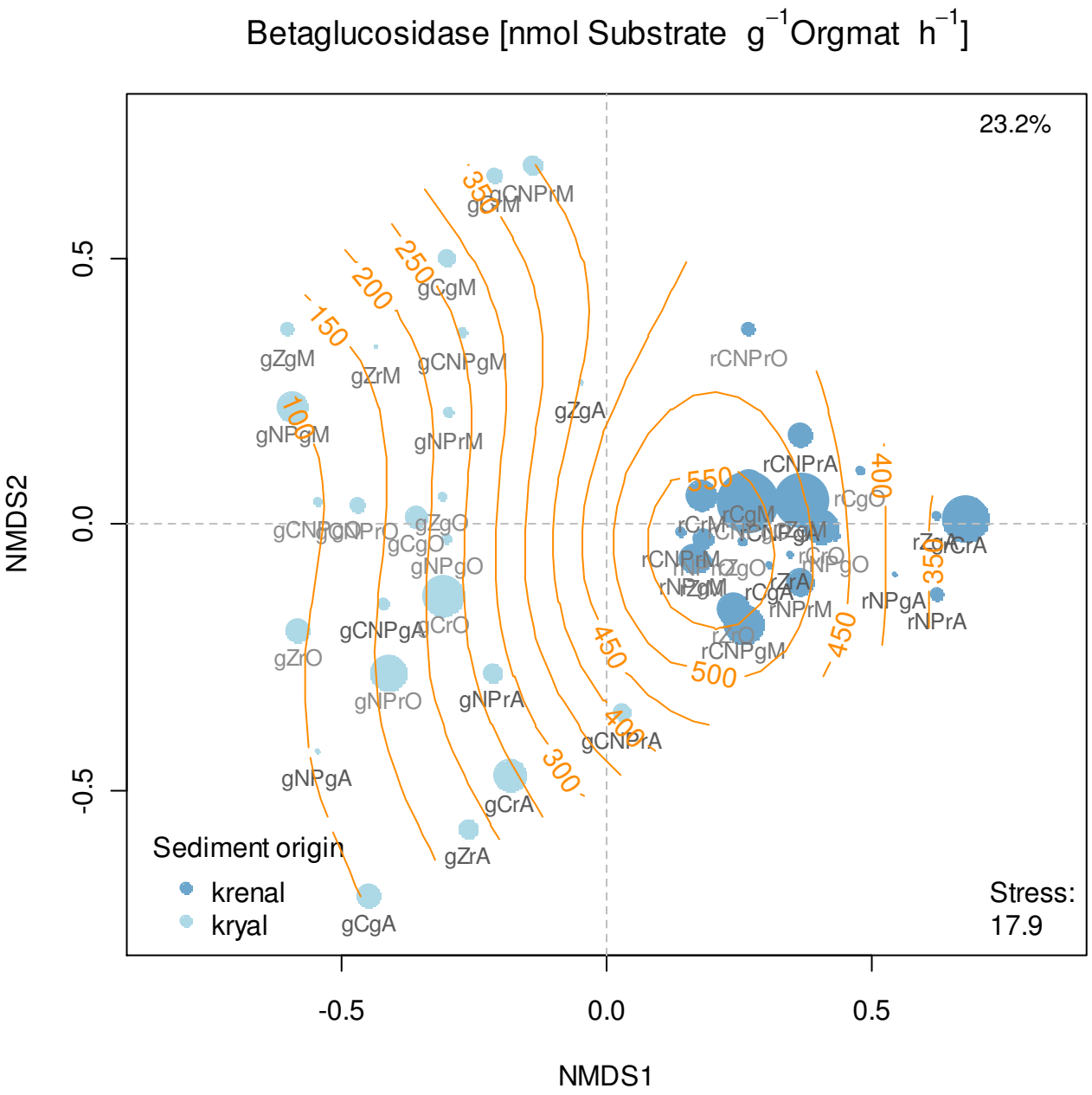
Appendix Figure 3b



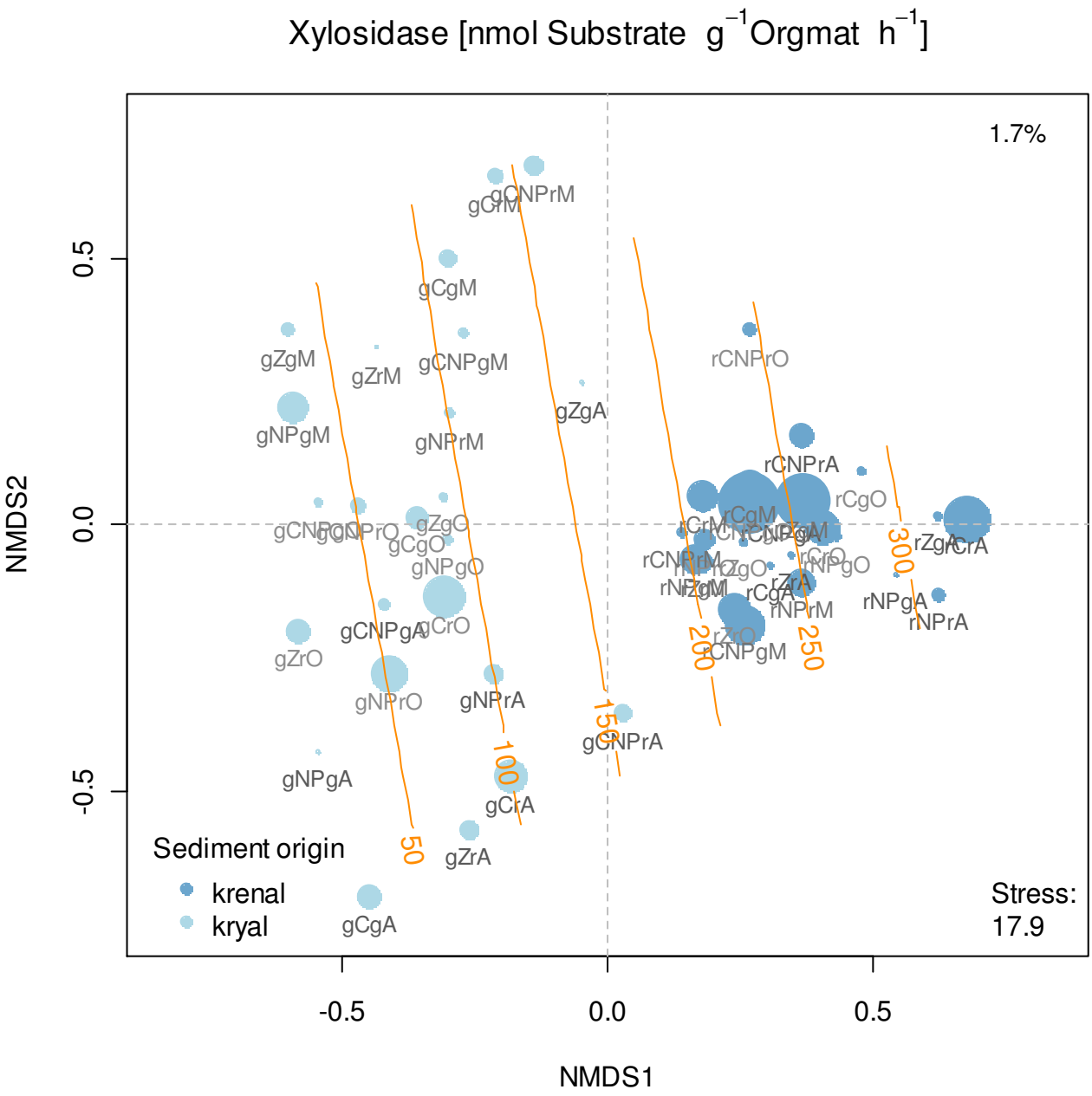
Appendix Figure 4a



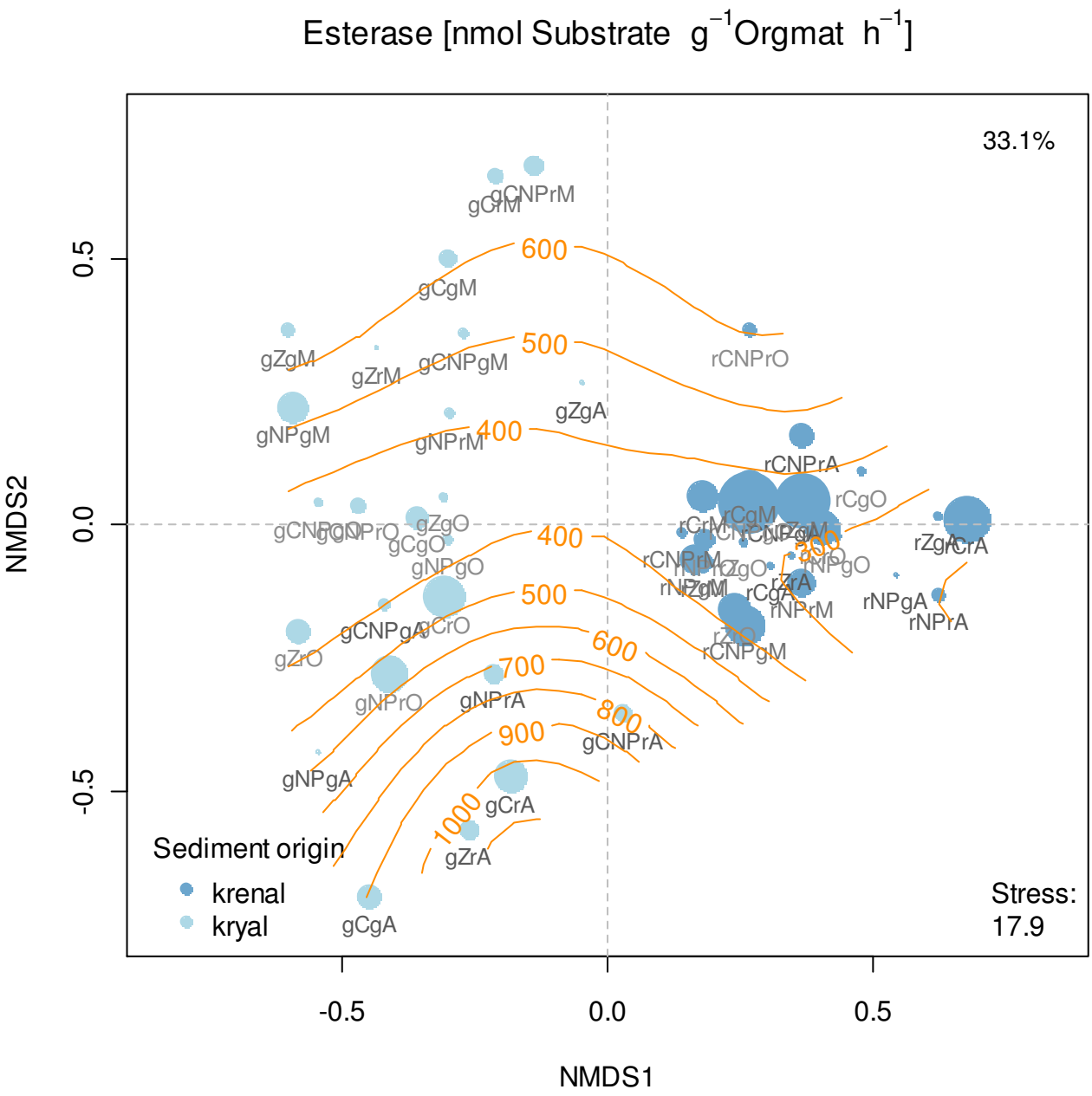
Appendix Figure 4b



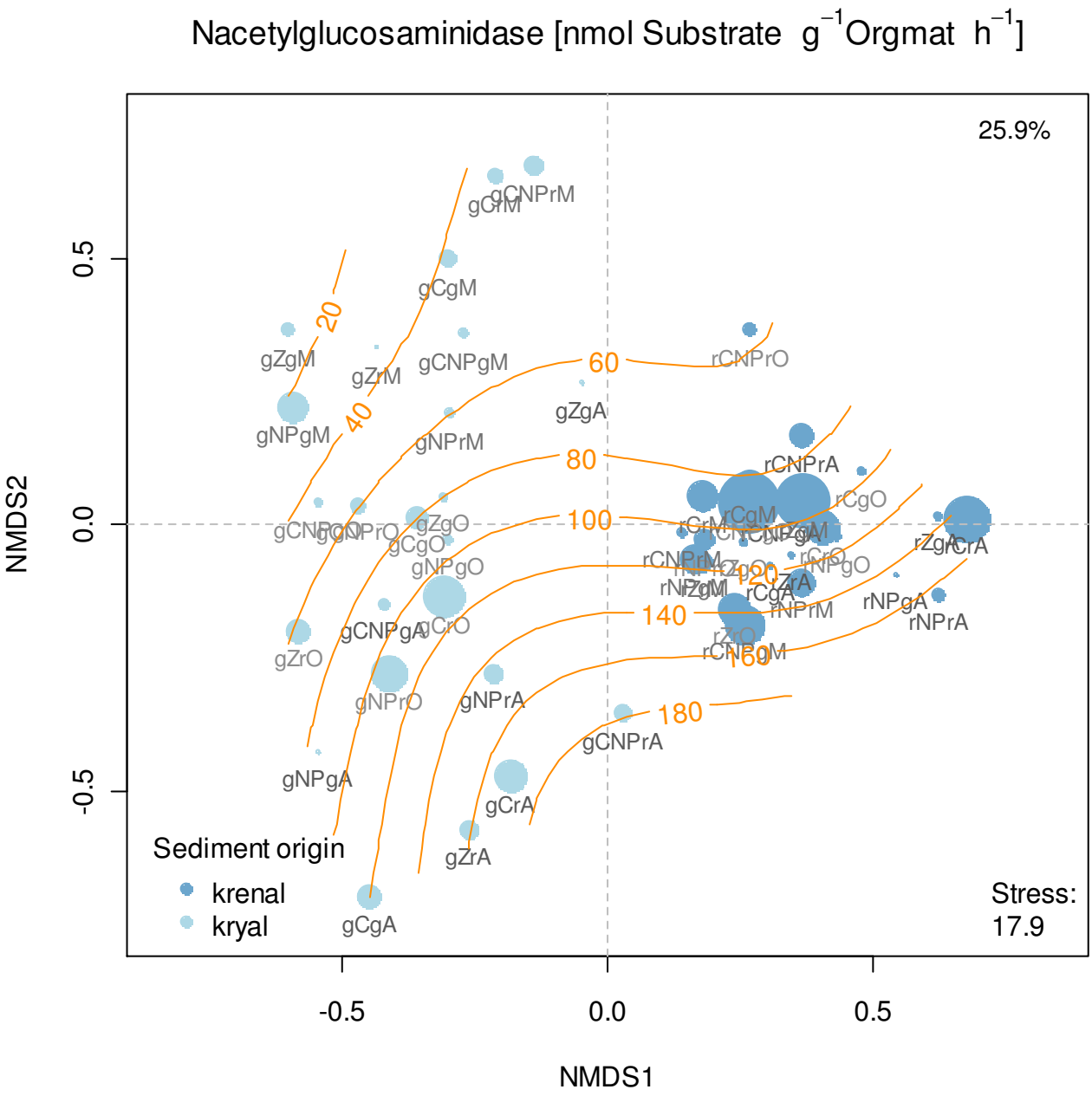
Appendix Figure 4c



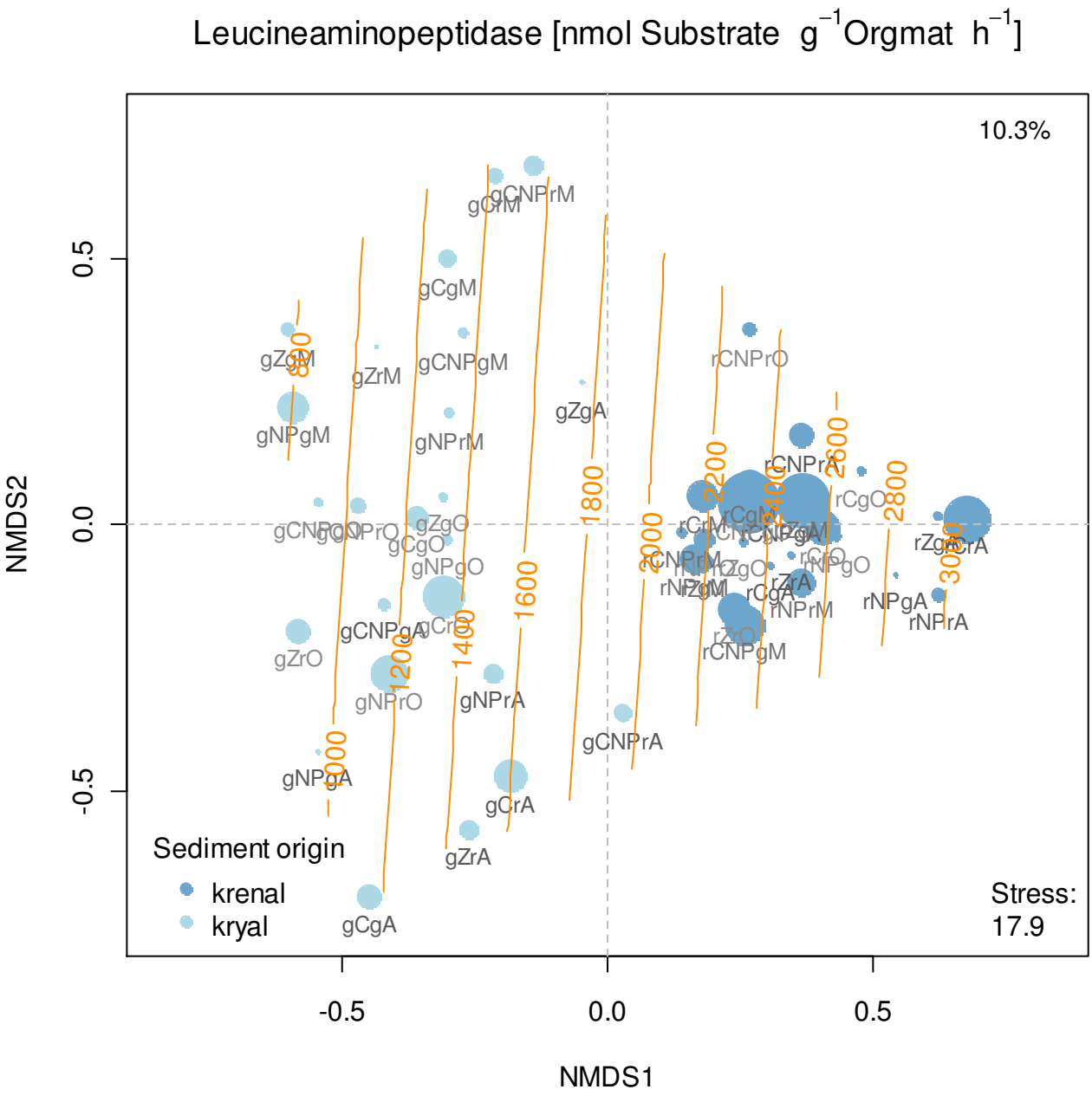
Appendix Figure 4d



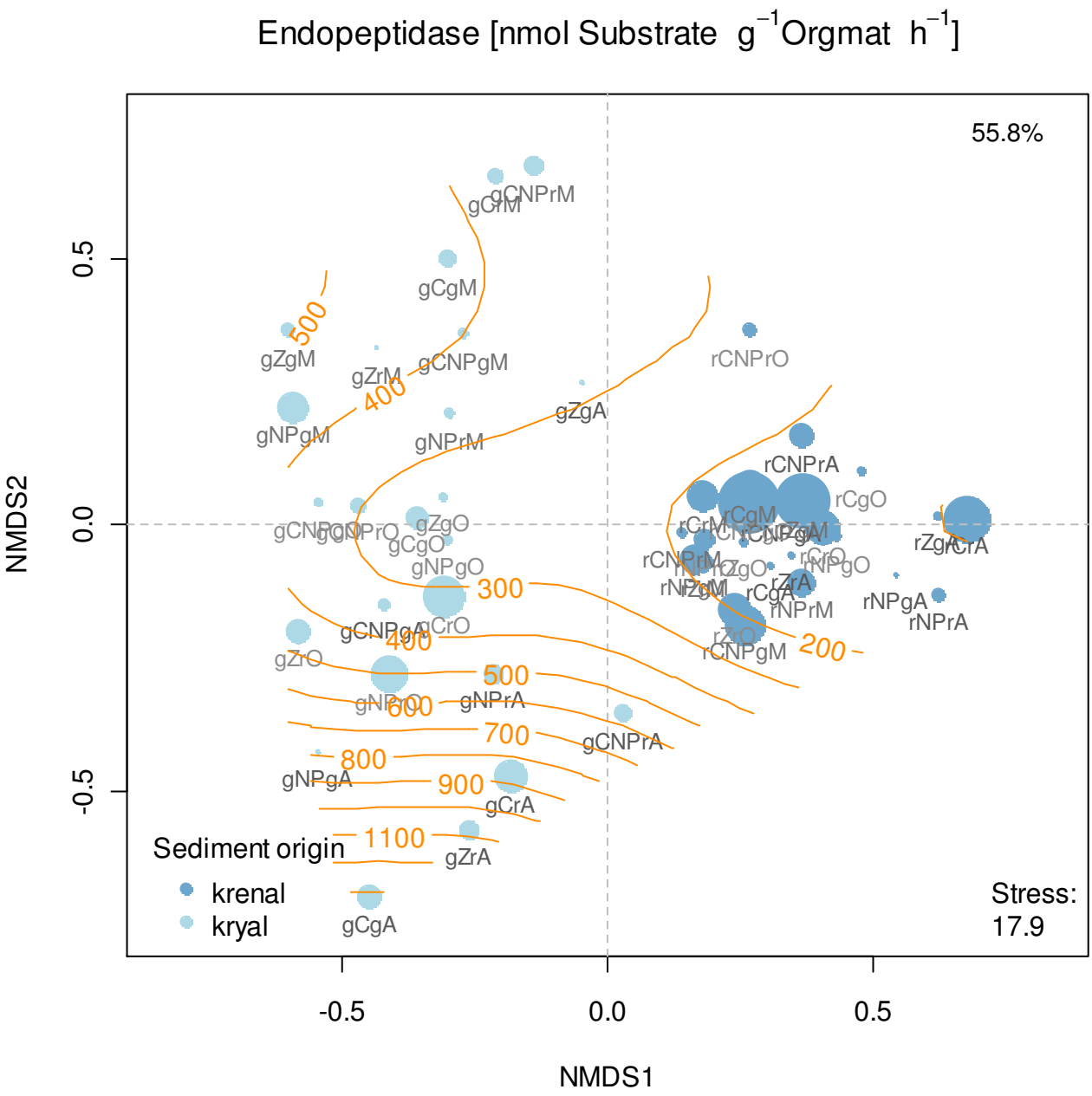
Appendix Figure 4e



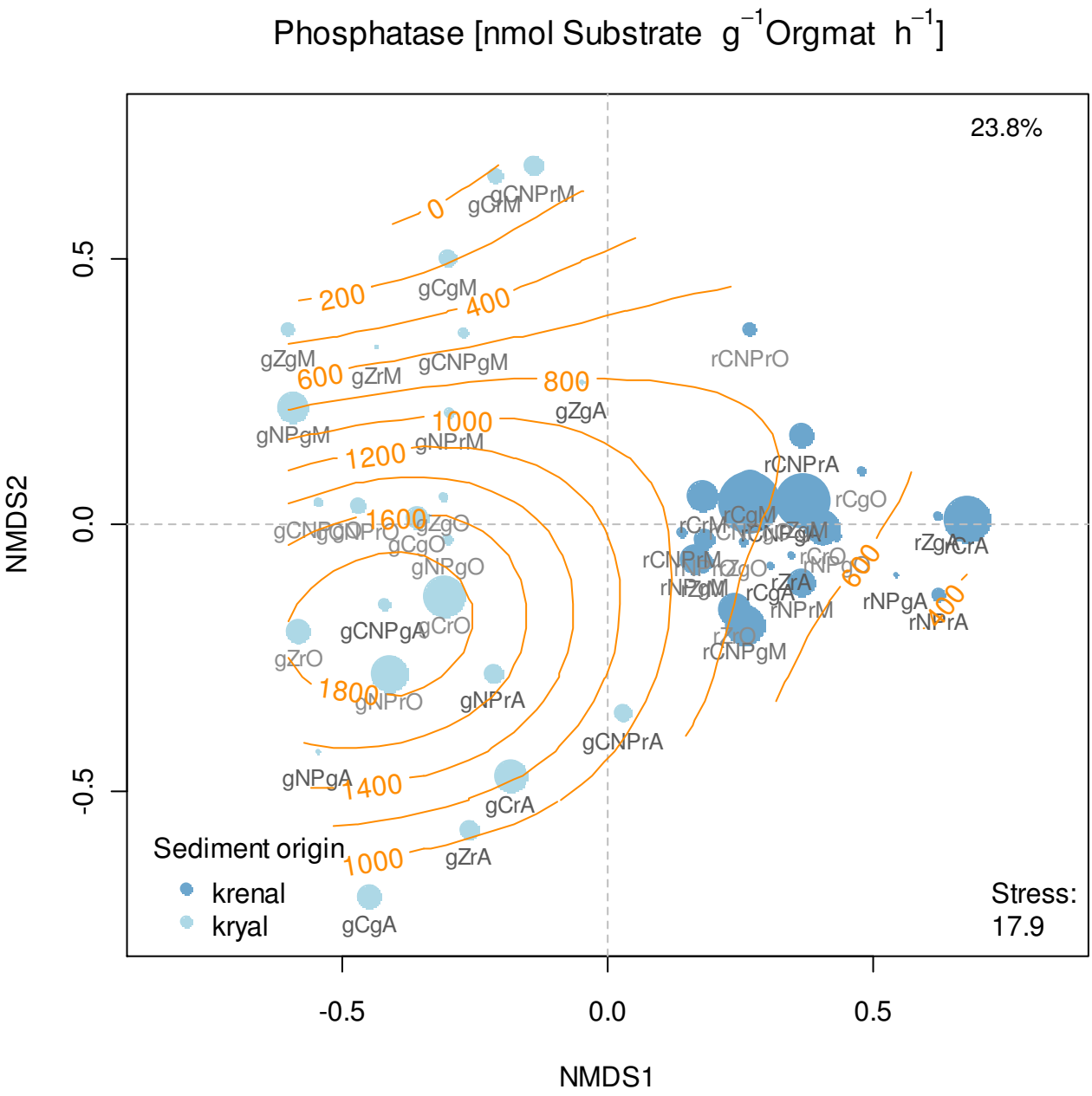
Appendix Figure 4f



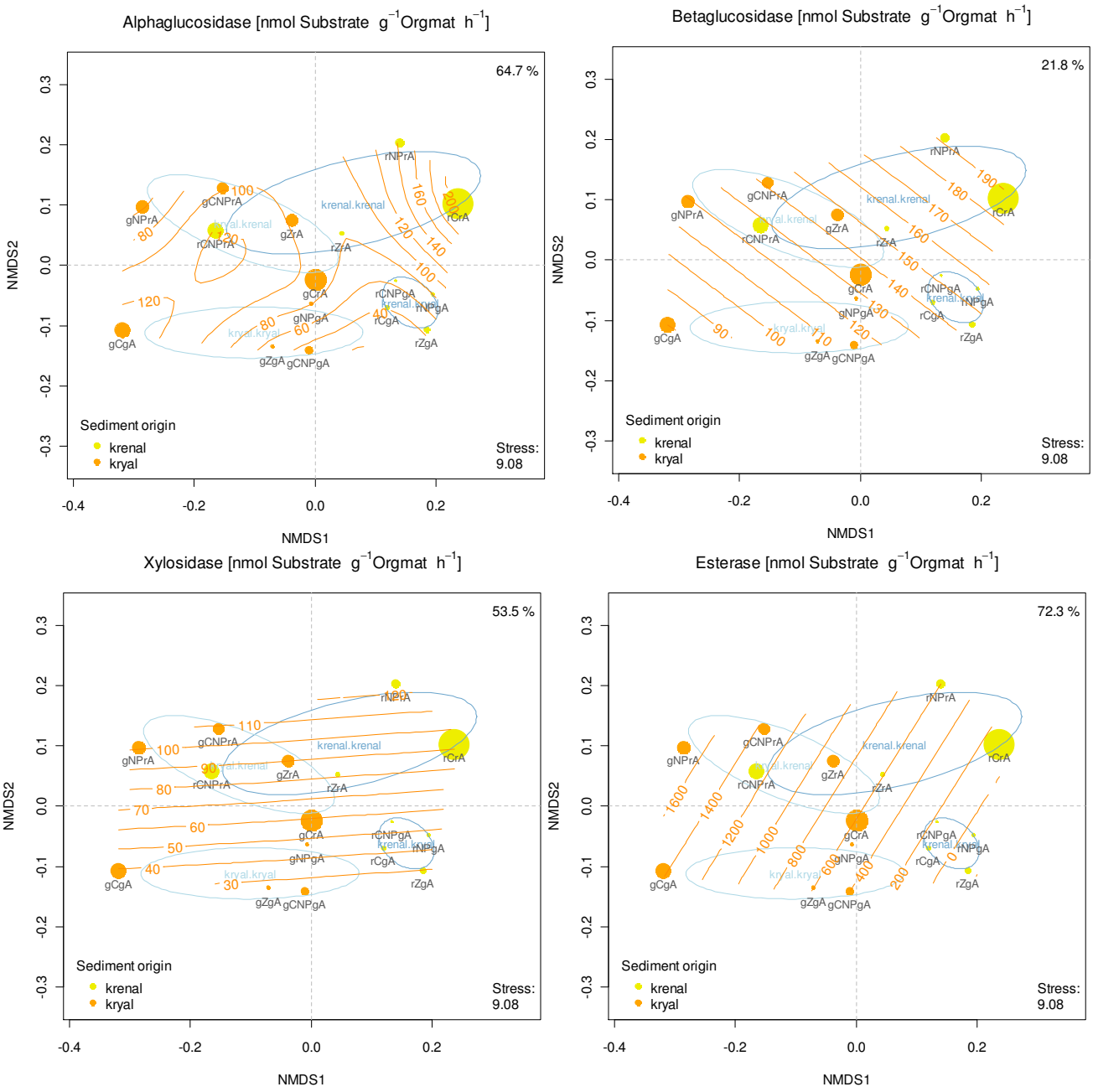
Appendix Figure 4g



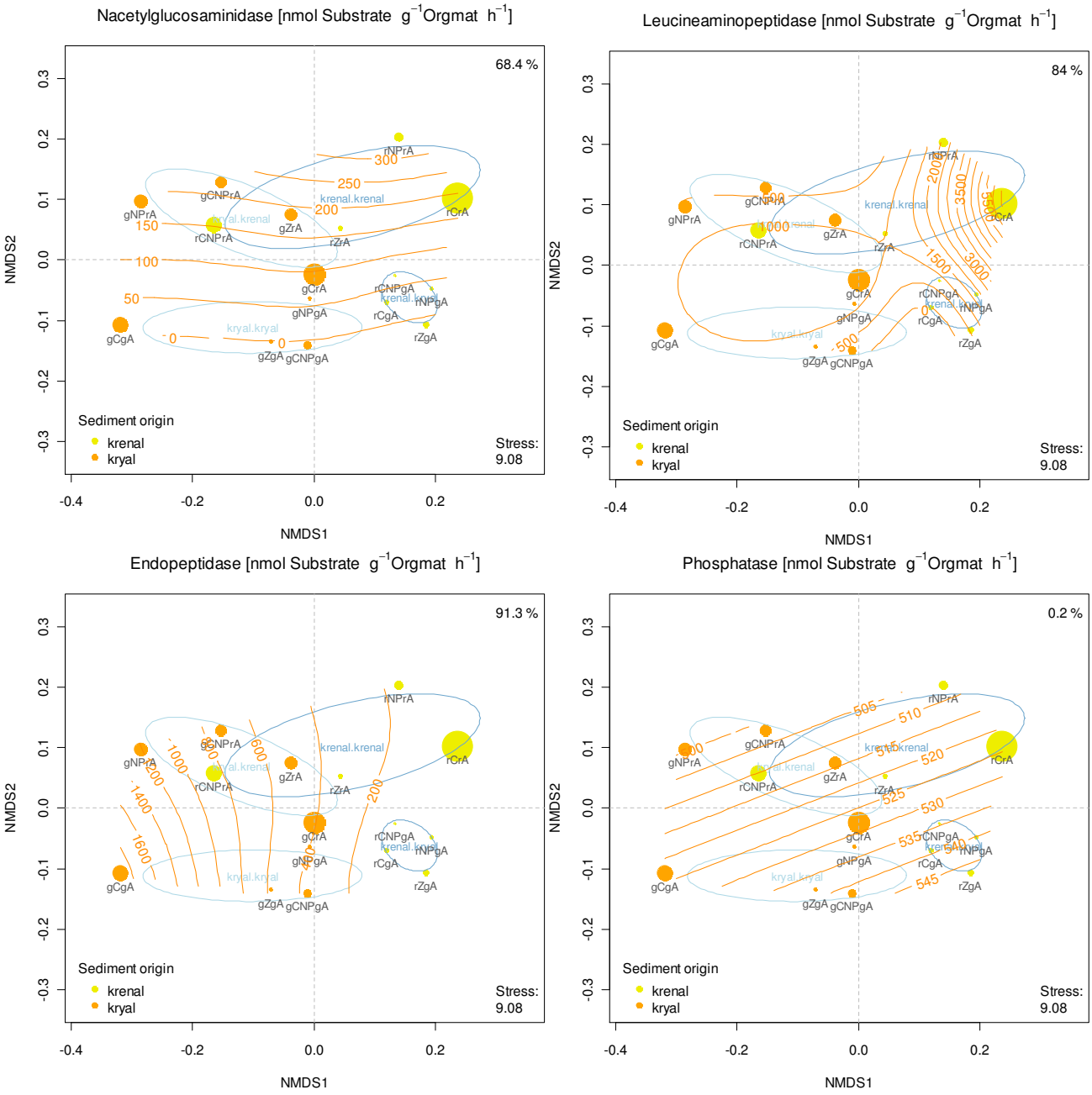
Appendix Figure 4h



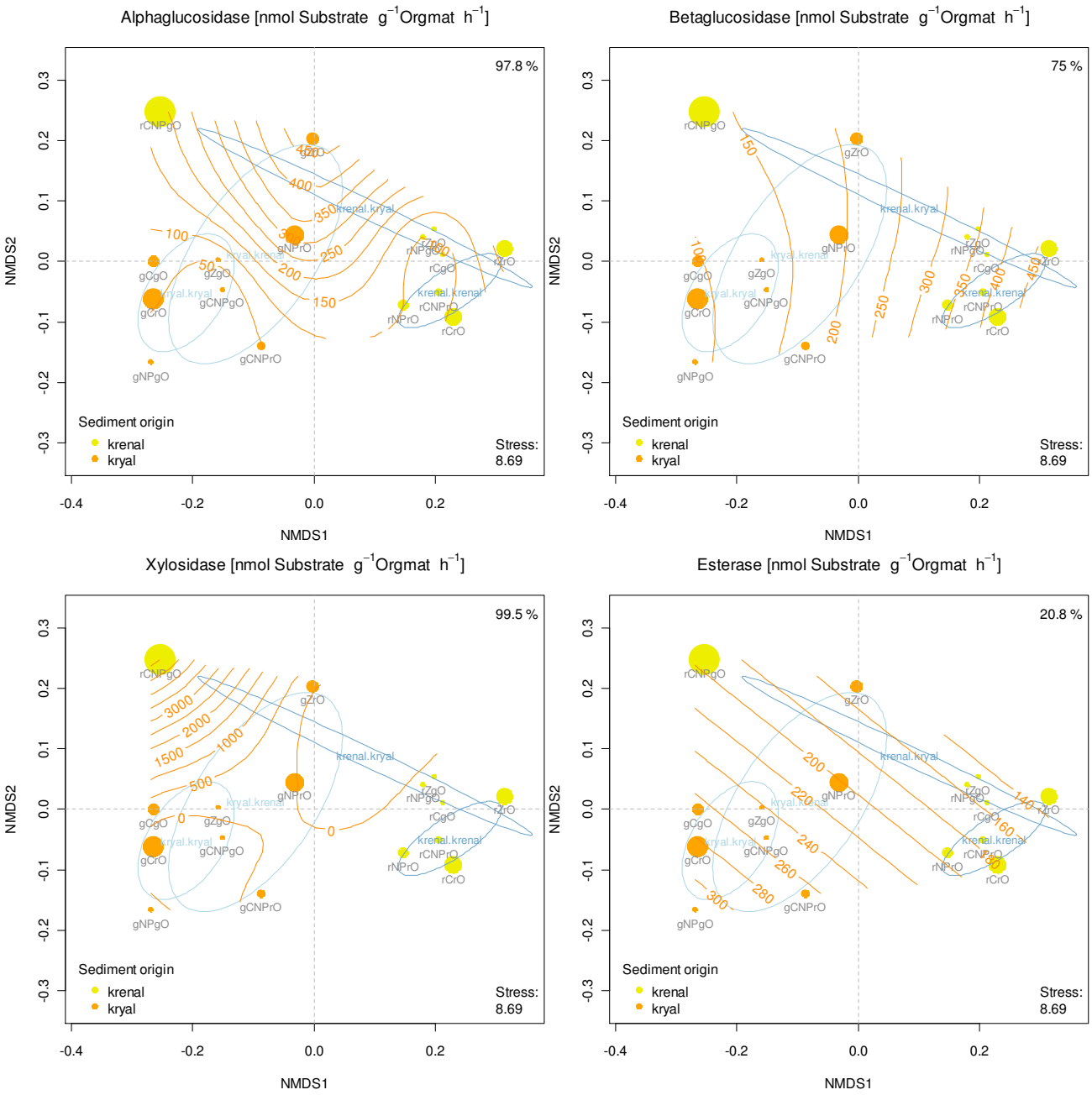
Appendix Figure 5a



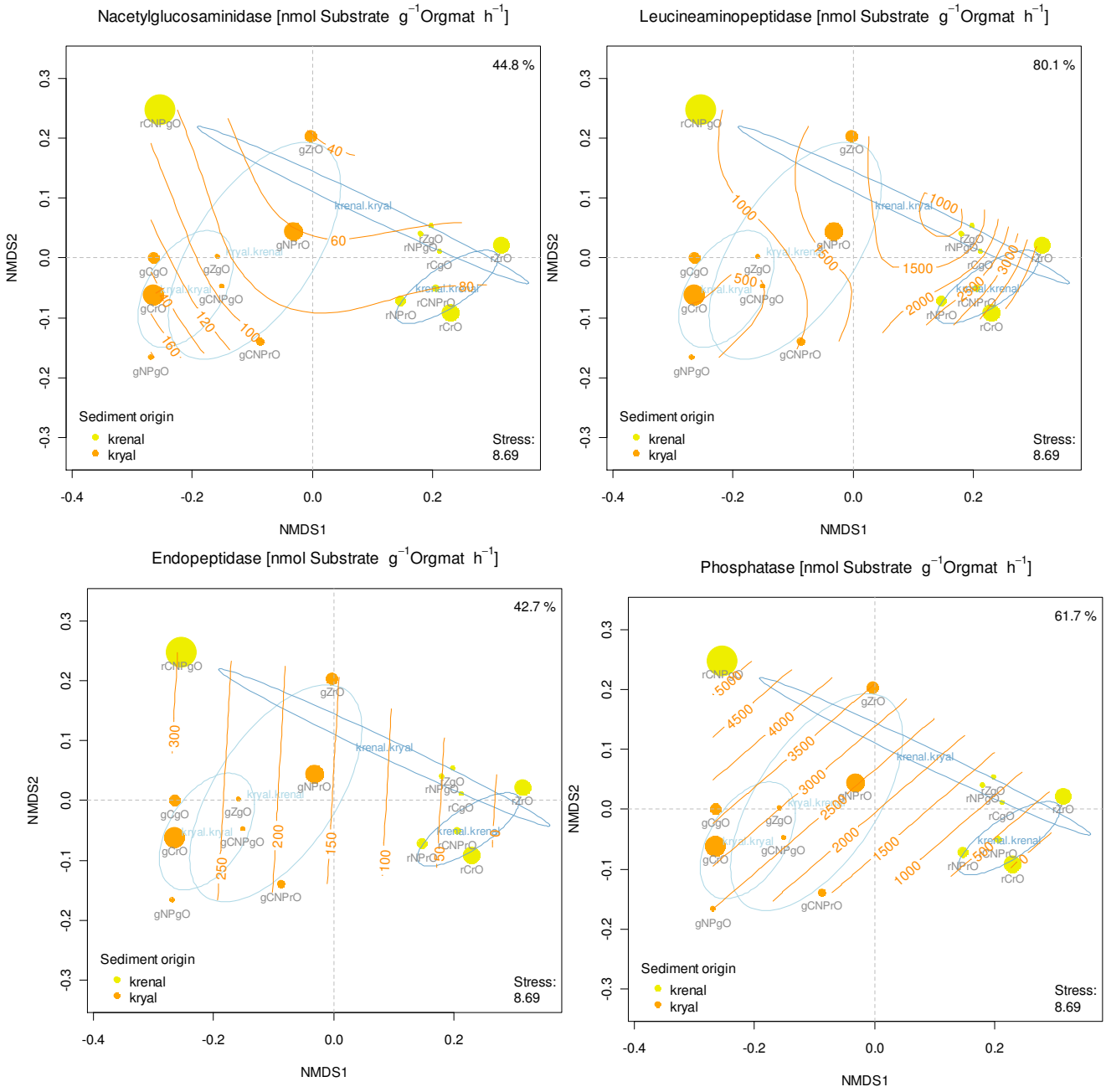
Appendix Figure 5b



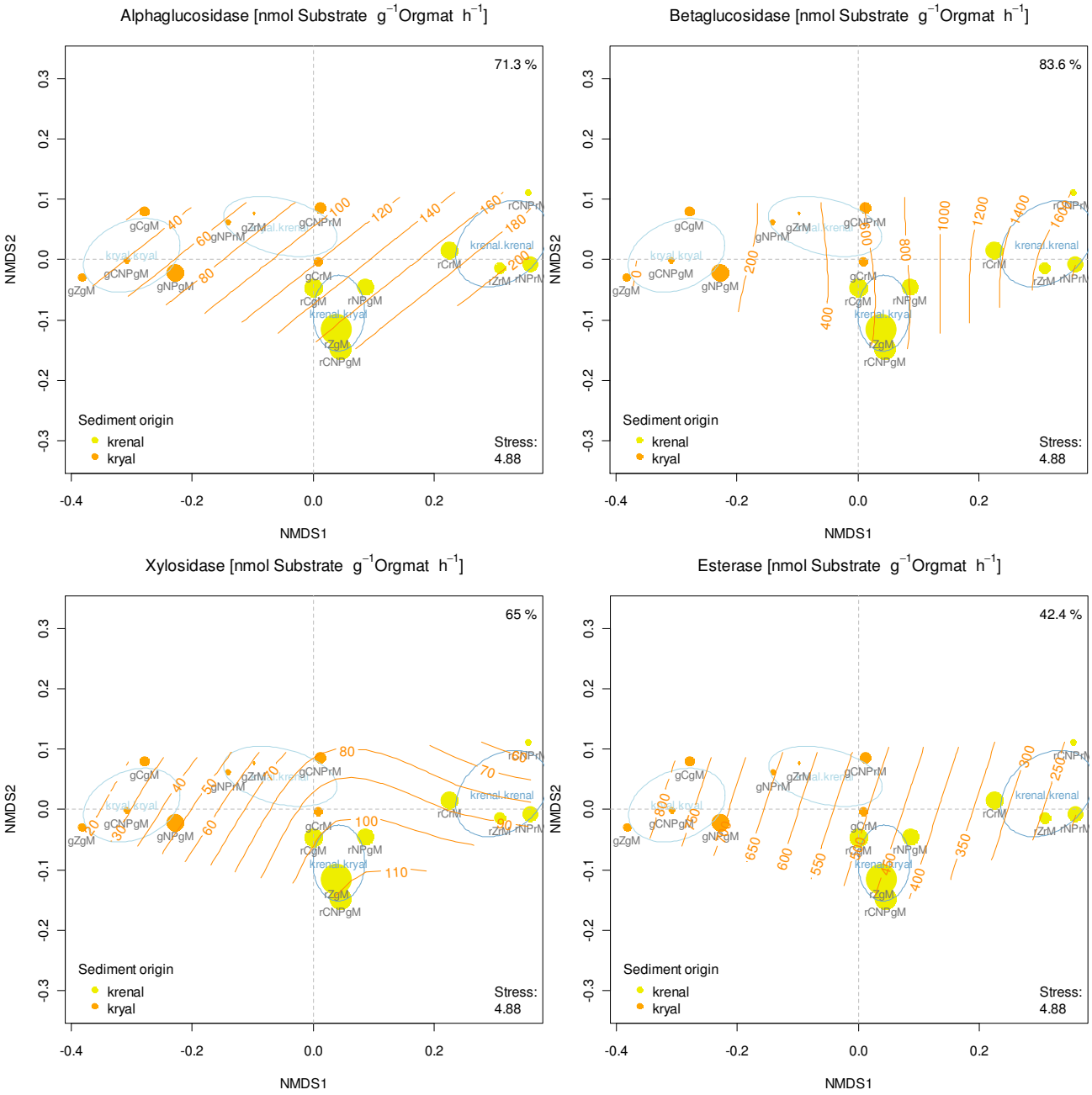
Appendix Figure 6a



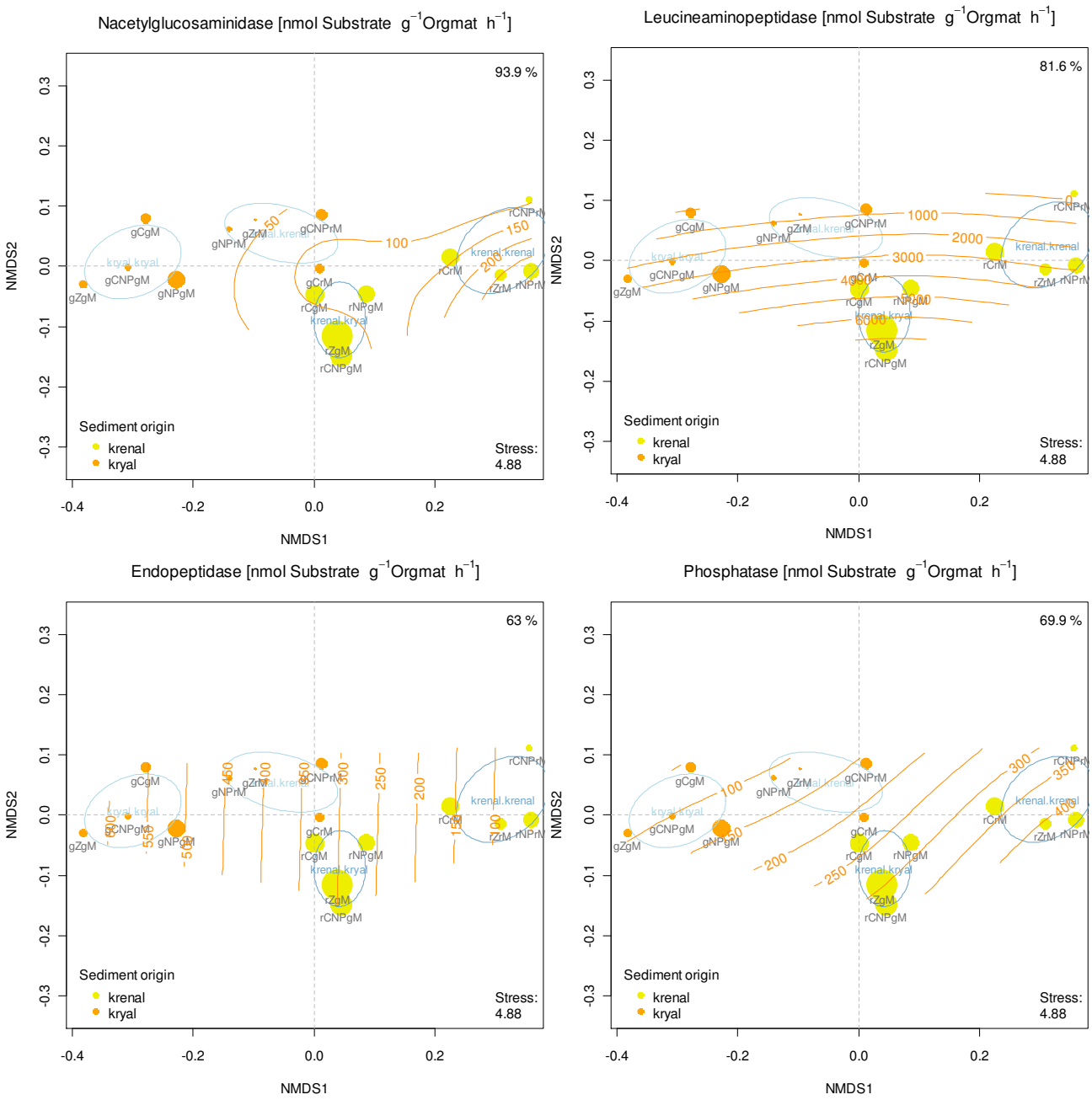
Appendix Figure 6b



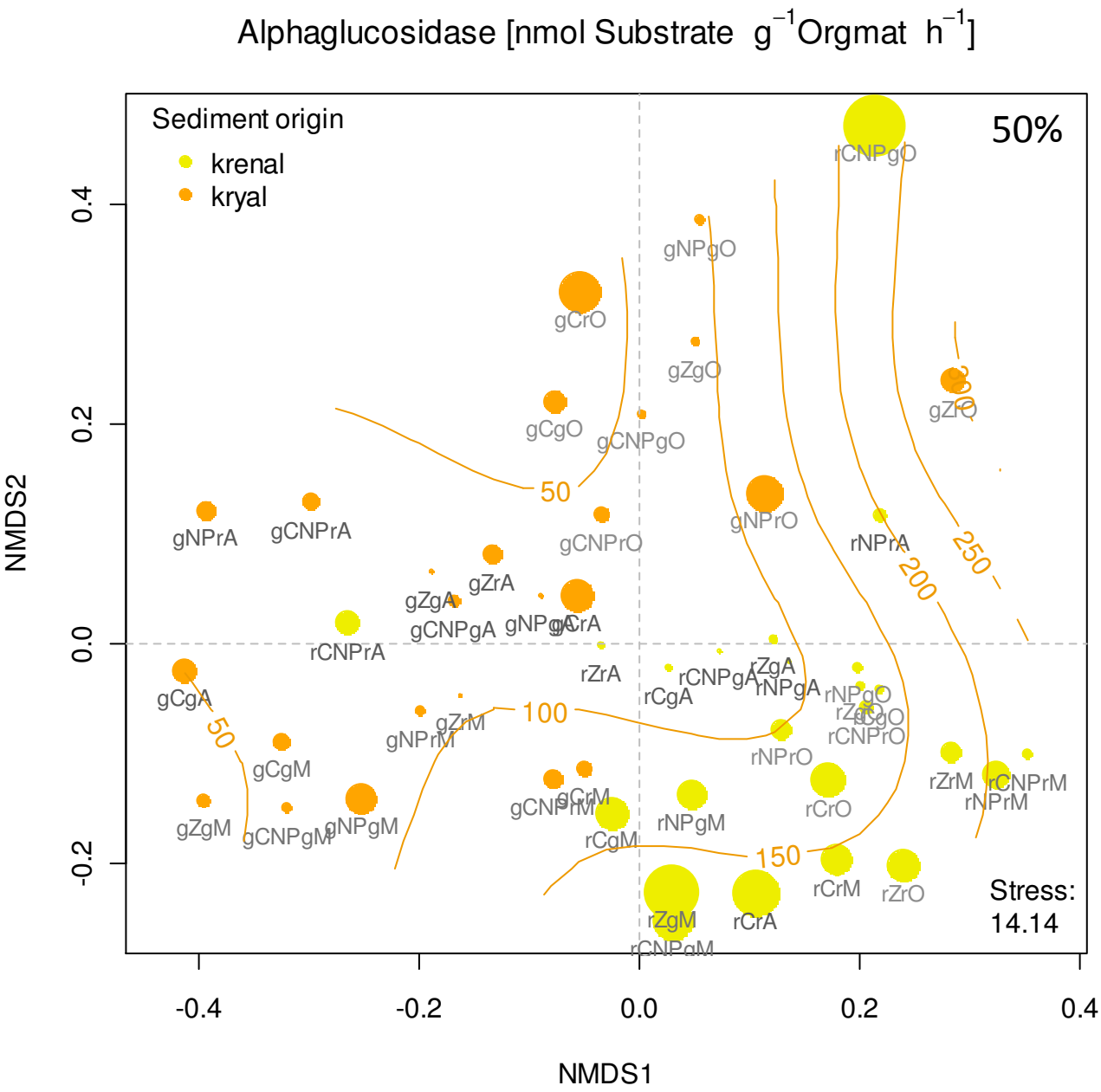
Appendix Figure 7a



Appendix Figure 7b



Appendix Figure 8a



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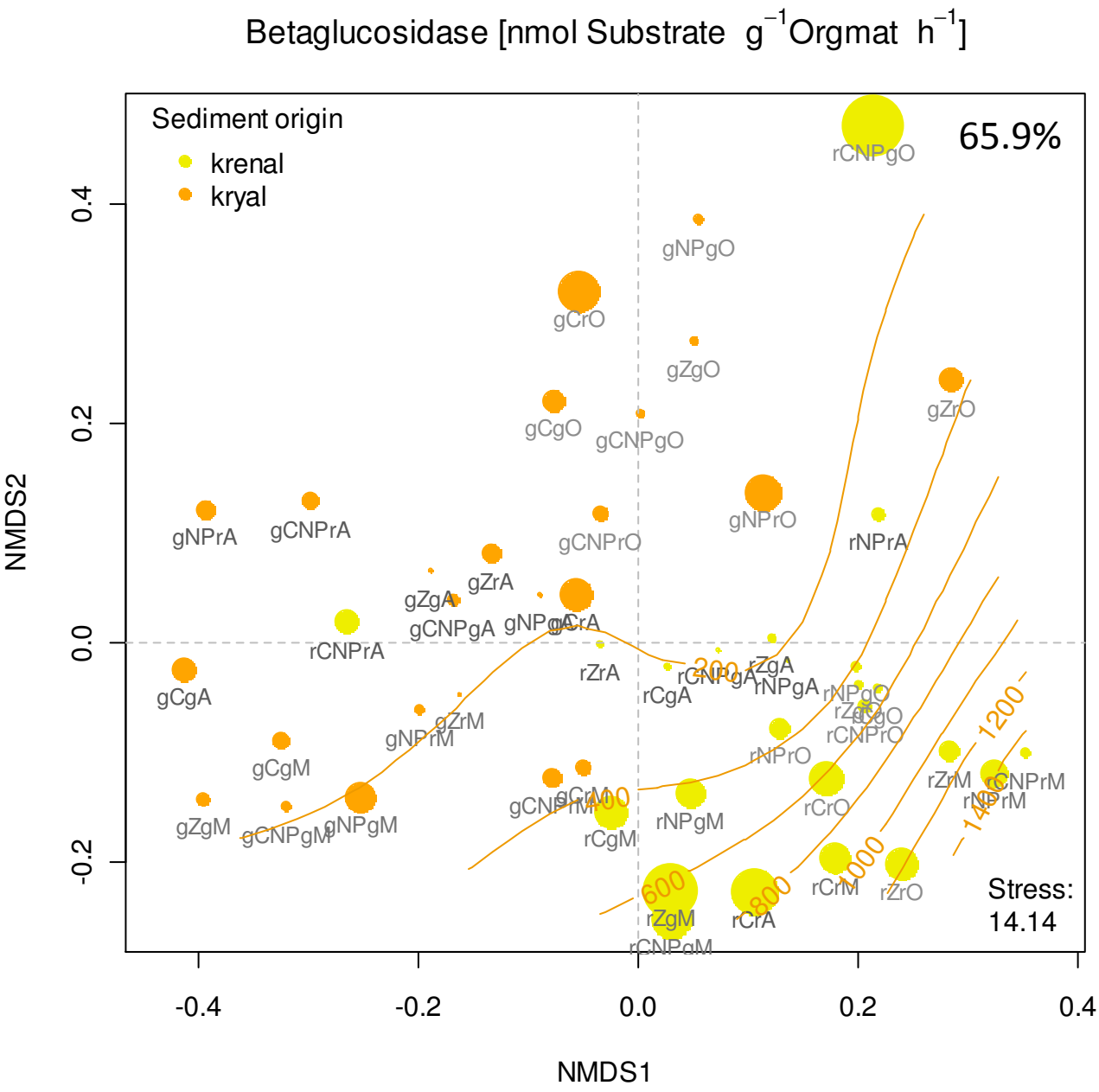
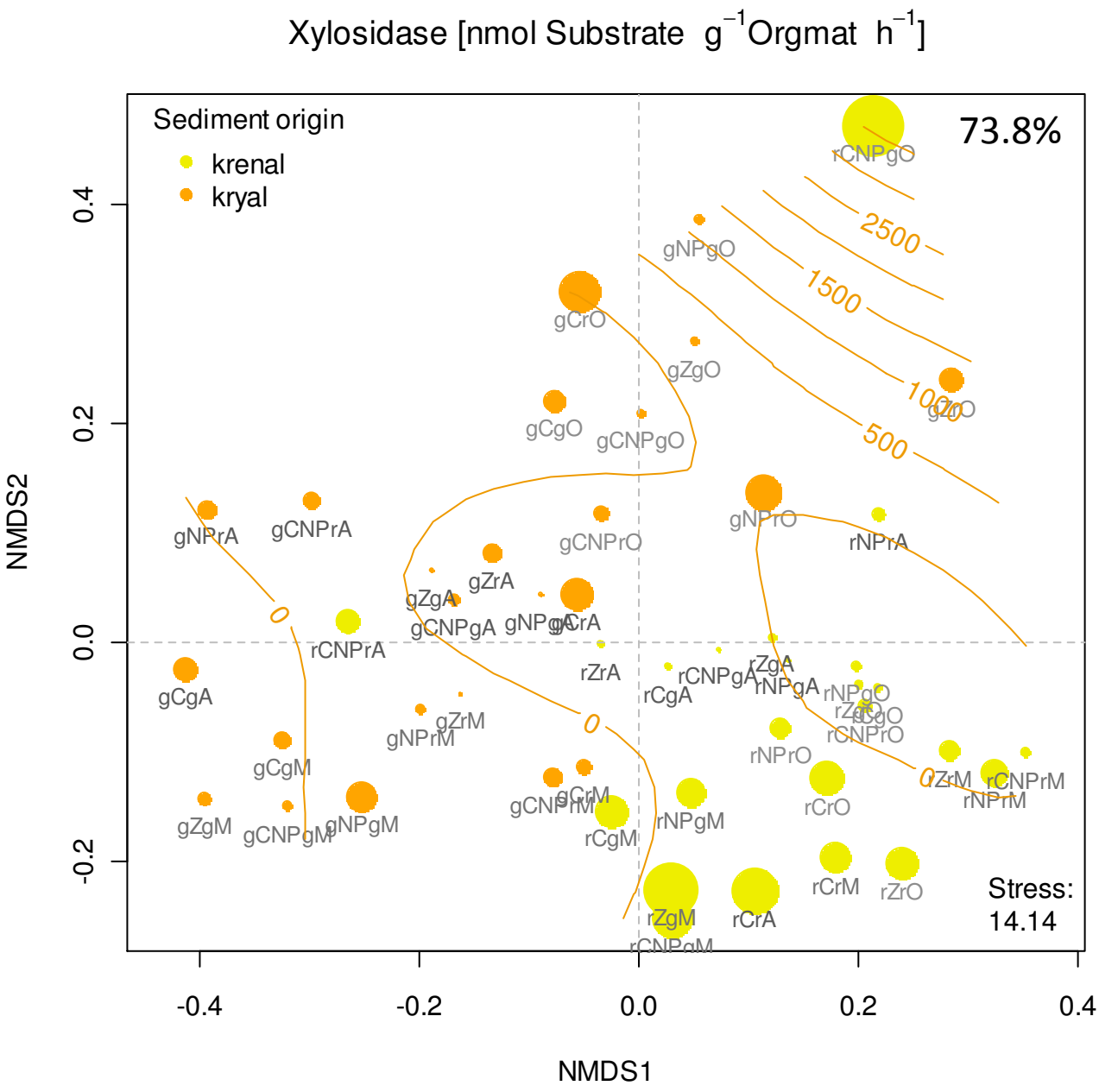
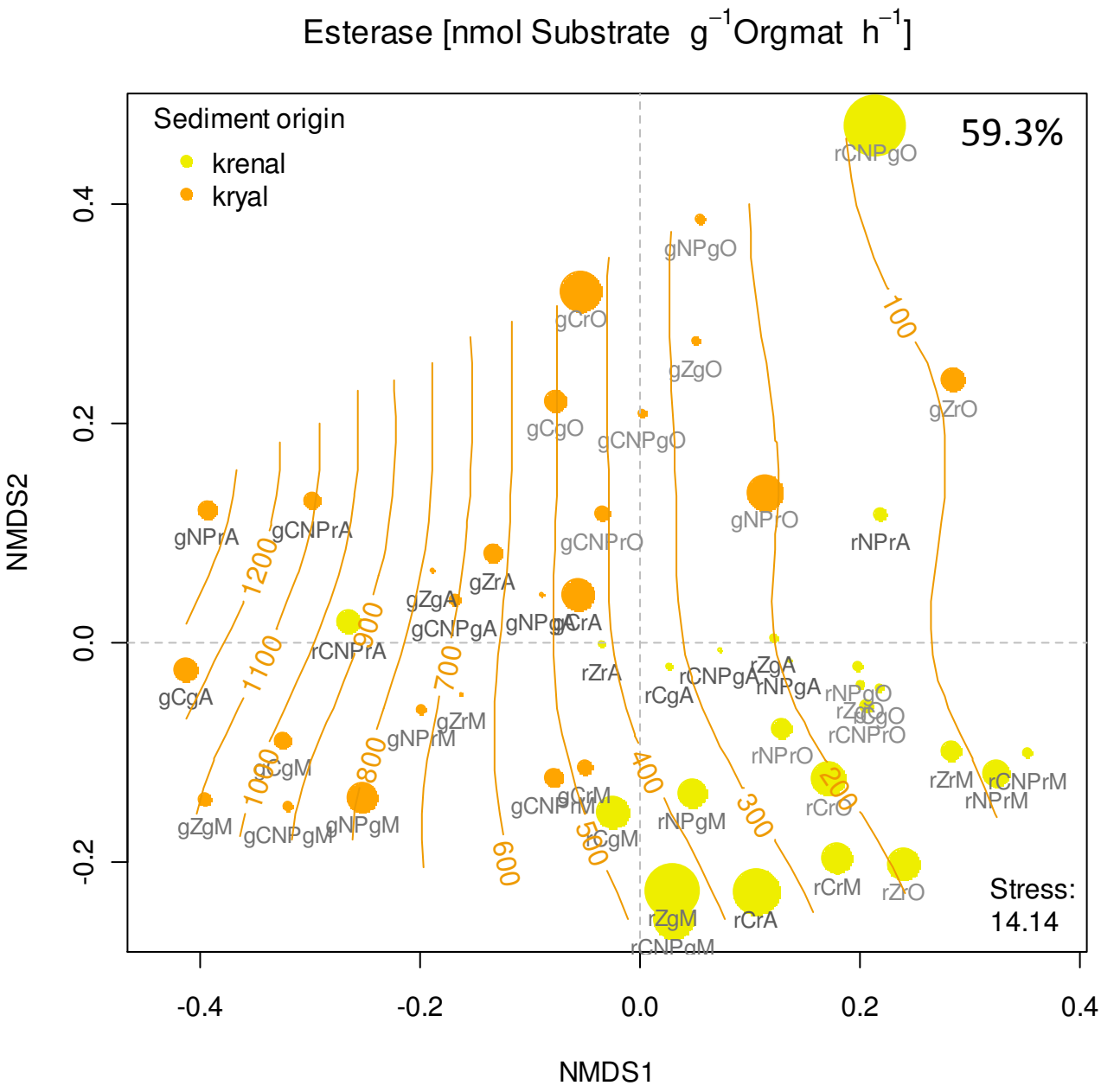


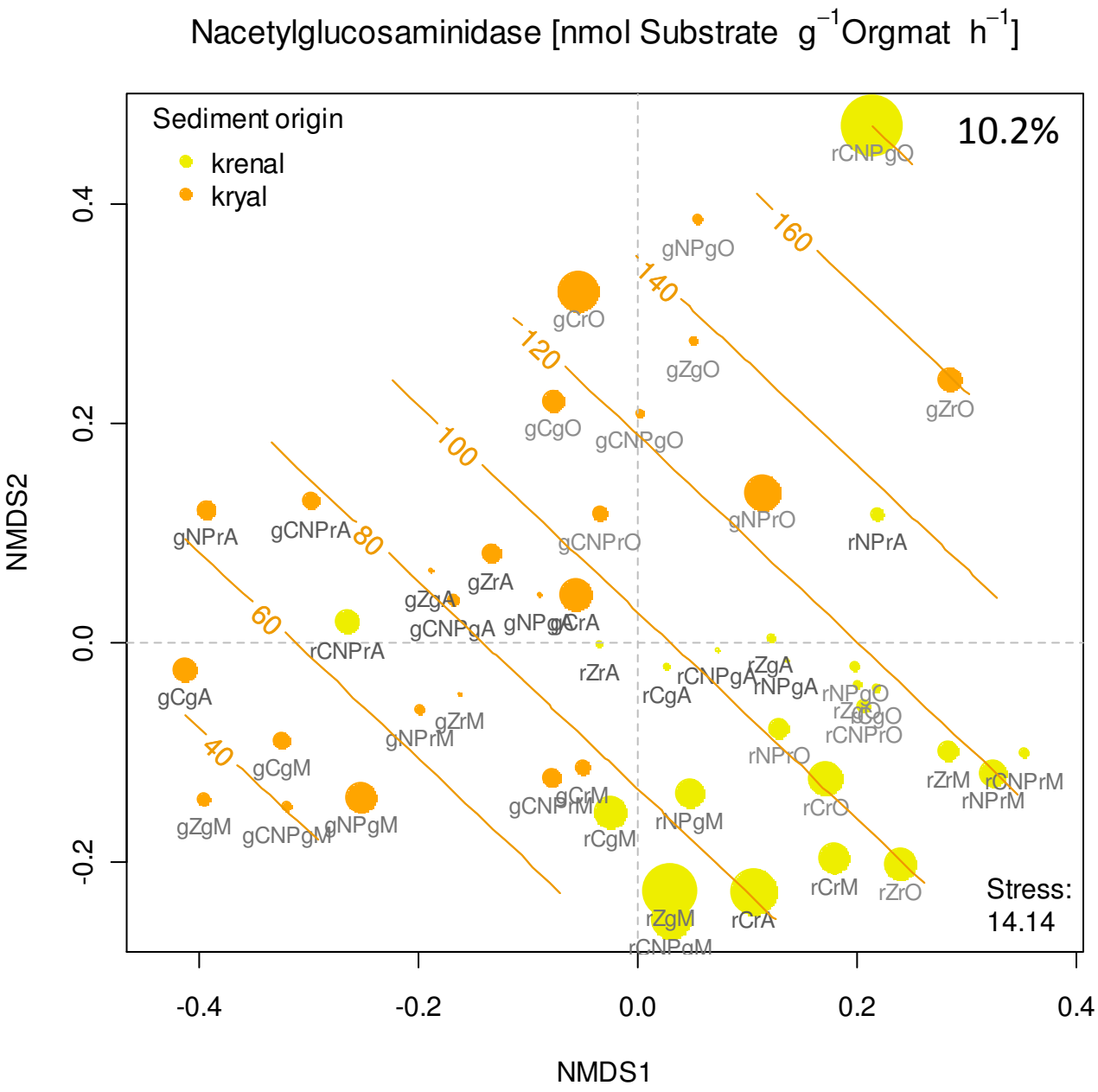
Appendix Figure 8c



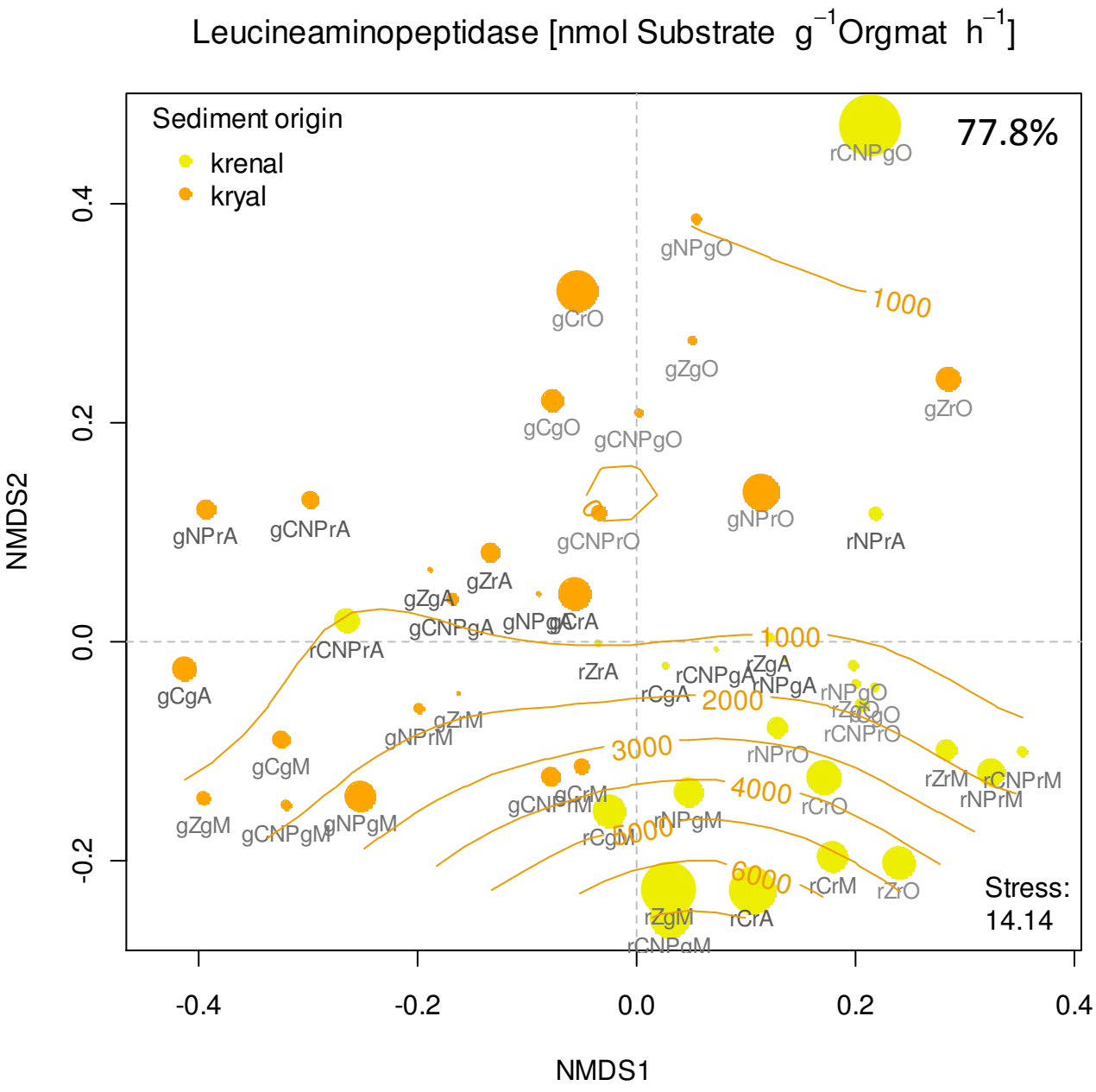
Appendix Figure 8d



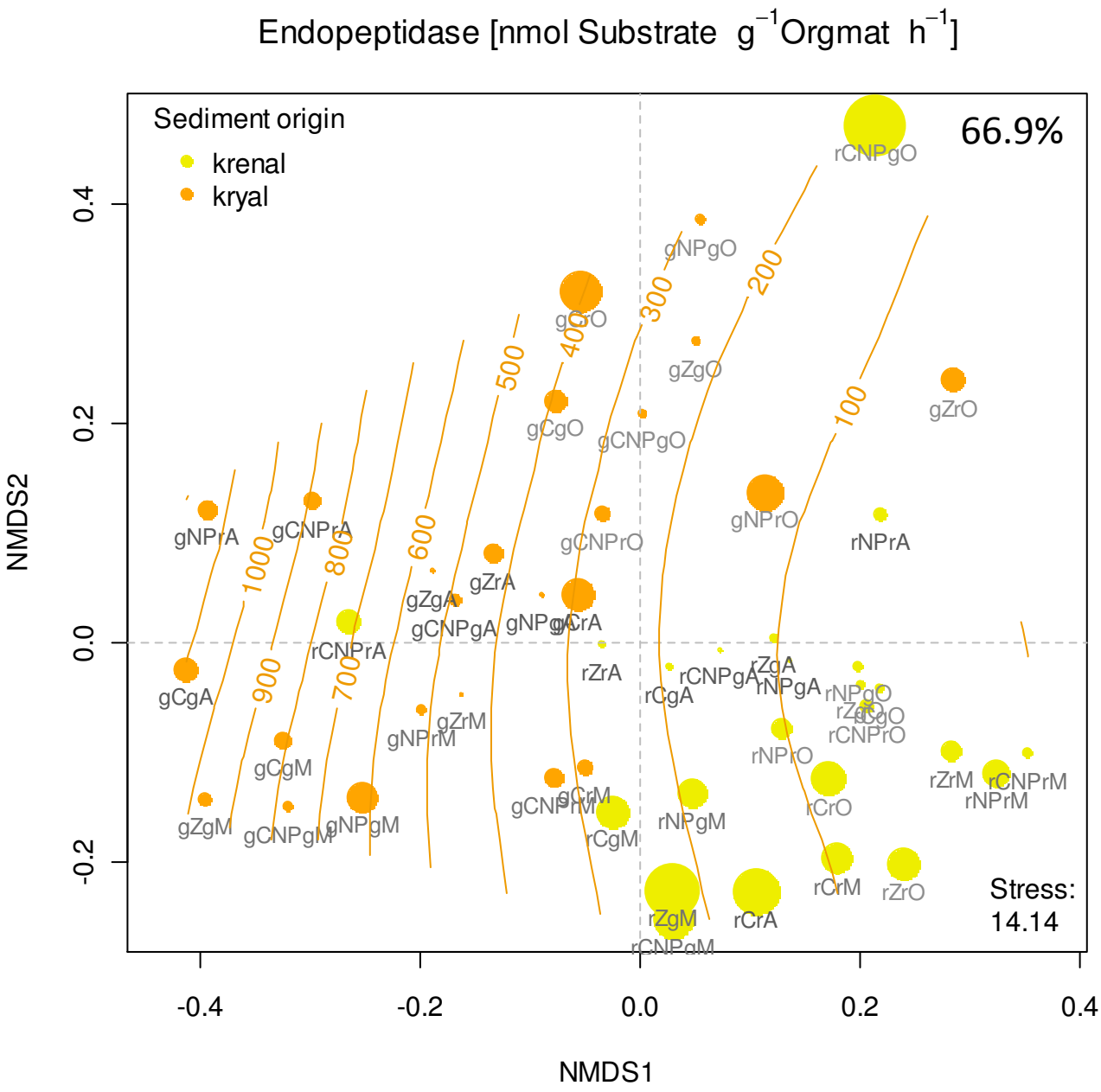
Appendix Figure 8e



Appendix Figure 8f



Appendix Figure 8g



Appendix Figure 8h

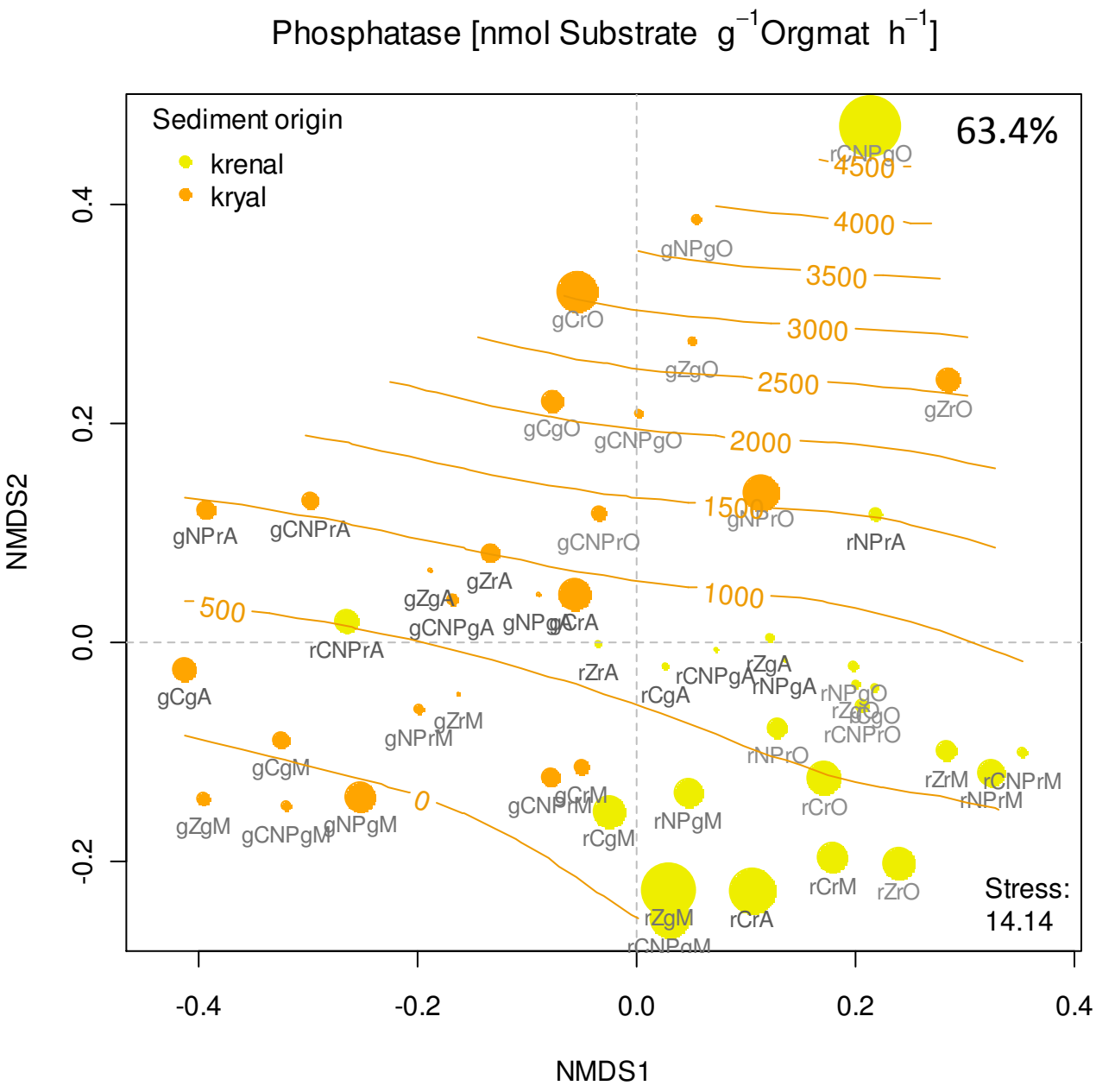


Table 1 Cell abundance mean values (± standard errors), differences between incubation sites and treatments were assessed by ANOVAs wihtin sediment types and seasons (Type III SS, terms: Treatment x Incubation Site, $dF_{tot,within}$=24,16) and followed by aTukey's HSD test (P<0.05) in case of significant treatment effect.

Sediment Origin			Krenal			
Season	Summer		Winter		Spring	
Incubation Site	krenal	kryal	krenal*	kryal*	krenal	kryal
Treatment						
Z	$4.32e^7(7.22e^6)^a$	$4.84e^7(1.68e^7)^a$	$1.04e^8(1.50e^7)^a$	$1.62e^8(9.02e^6)^b$	$1.79e^8(1.14e^8)^{ab}$	$1.49e^8(2.32e^7)^{ab}$
C	$7.17e^7(4.46e^6)^a$	$4.13e^7(3.25e^7)^a$	$8.05e^7(1.61e^7)^{ac}$	$6.66e^7(2.99e^6)^c$	$7.57e^7(4.61e^7)^{ab}$	$4.55e^7(2.01e^7)^b$
NP	$5.13e^7(1.03e^7)^a$	$5.59e^7(9.80e^6)^a$	$1.24e^8(2.62e^7)^{ab}$	$4.69e^7(2.24e^6)^c$	$1.98e^8(1.77e^7)^a$	$1.39e^8(6.14e^6)^{ab}$
CNP	$4.27e^7(9.20e^6)^a$	$4.39e^7(2.10e^7)^a$	$1.53e^8(2.00e^7)^b$	$3.01e^7(1.70e^6)^d$	$1.87e^8(1.21e^8)^{ab}$	$2.12e^8(9.13e^7)^a$
Sediment Origin			Kryal			
Season	Summer		Winter		Spring	
Incubation Site	krenal	kryal	krenal*	kryal*	krenal*	kryal*
Treatment						
Z	$1.53e^7(3.28e^6)^a$	$8.91e^6(2.26e^6)^a$	$8.35e^6(1.79e^6)^a$	$9.43e^6(1.06e^6)^{ae}$	$1.13e^7(6.15e^6)^{ab}$	$4.59e^6(1.09e^6)^a$
C	$1.36e^7(2.65e^6)^a$	$1.40e^7(3.49e^6)^a$	$2.21e^7(6.28e^6)^b$	$1.08e^7(2.99e^6)^{ae}$	$3.75e^7(2.81e^7)^b$	$8.14e^6(1.72e^6)^{abc}$
NP	$1.72e^7(7.17e^6)^a$	$1.25e^7(4.82e^6)^a$	$1.48e^8(2.47e^7)^c$	$9.21e^6(4.75e^5)^{ae}$	$1.49e^7(1.32e^7)^{abc}$	$4.34e^6(1.30e^6)^{ab}$
CNP	$1.42e^7(2.13e^6)^a$	$1.24e^7(3.90e^6)^a$	$5.92e^7(1.62e^7)^d$	$1.66e^7(3.25e^6)^{be}$	$2.90e^7(1.43e^7)^c$	$1.12e^7(2.67e^6)^{abc}$

a-e group allocation due to treatment effect (Tukey's HSD P<0.05)

* significant effect of incubation site (Tukey's HSD P<0.05)

Table 2 Complete PERMANOVA model of ARISA profiles (n=2, df=95). Treatment (T), Incubation site (IS), season (S) and sediment origin (SO) were used as fixed factors.

Factor	dF	F	R^2	P
Treatment (T)	3	1.54	0.03	0.011
Incubation site (IS)	1	2.01	0.01	0.013
Season (S)	2	6.57	0.08	<0.001
Sediment origin (SO)	1	27.04	0.17	<0.001
T x IS	3	1.35	0.03	0.047
T x S	6	1.57	0.06	0.001
IS x S	2	1.67	0.02	0.012
T x SO	3	1.49	0.03	0.019
IS x SO	1	1.31	0.01	0.143
S X SO	2	6.74	0.08	<0.001
T x IS x S	6	1.24	0.05	0.056
T x IS x SO	3	1.31	0.02	0.068
T x S x SO	6	1.22	0.05	0.068
IS x S x SO	2	1.37	0.02	0.071
T x IS x S x SO	6	1.35	0.05	0.016

Table 3 Complete PERMANOVA model of enzymatic activities patterns (n=3, dF=143). Treatment (T), Incubation Site (IS), Season (S) and Sediment Origin (SO) were used as fixed factors.

Factor	dF	F	R^2	P
Treatment (T)	3	1.67	0.01	0.081
Incubation site (IS)	1	6.48	0.02	<0.001
Season (S)	2	40.35	0.19	<0.001
Sediment origin (SO)	1	113.57	0.27	<0.001
T x IS	3	2.52	0.02	0.009
T x S	6	0.96	0.01	0.507
IS x S	2	21.26	0.1	<0.001
T x SO	3	0.57	0	0.857
IS x SO	1	1.60	0	0.173
S X SO	2	11.69	0.06	<0.001
T x IS x S	6	1.77	0.03	0.034
T x IS x SO	3	1.42	0.01	0.156
T x S x SO	6	1.50	0.02	0.072
IS x S x SO	2	2.79	0.01	0.014
T x IS x S x SO	6	0.63	0.01	0.908

5 Discussion, conclusion and outlook

5.1 General remarks and conceptual background

The present thesis investigated microbial communities within alpine floodplains and revealed different mechanisms driving microbial assemblage, maintenance, spatio-temporal dynamics and microbial mediated functions. Although these ecosystems represent an important contributor for freshwater intensively used by humans in lower elevation regions, this dataset is one of the first to include different spatial and temporal scales concerning bacterial community composition and functions covering different habitats. There are few studies dealing with bacteria in glaciated floodplains and those in existence mostly investigate soils (i.e. Battin *et al.,* 2004, Hämmerli *et al.,* 2007, Noll and Wellinger 2008, Duc *et al.,* 2009, Philippot *et al.,* 2011, Lazzaro *et al.,* 2012). The results give insight in potential bacterial communities and functional responses to future landscape transformations.

The underlying conceptual idea of the presented thesis can be visualized as a linkage of different factors driving bacterial dynamics and ecosystem functioning. More specifically, terrestrial and aquatic bacterial communities are linked through subsurface and surface hydrological flow paths. These hydrological linkages mediate a partially directional exchange of nutrients and cells. Hydrology, in turn, is coupled to the water cycle, which is influenced by climate (i.e. precipitation patterns or presence of glaciers). Biogeochemical properties of the water cycle depend on landscape characteristics and structures such as geology (i.e. weathering influence on water chemistry) or strength in connectivity between streams and surrounding soils (i.e. leaching of organic matter). Hydrology links different biota and mediates timing, quantity and quality of resources, thus finally driving ecosystem functioning. Changes in ecosystem functioning, on the other hand, are directly related to the ecological services that alpine areas provide humans and downstream receiving waters. These changes in ecosystem services can be related back to microbes, as microbes are the essential integrators in the functioning of most ecosystems. Longer-term ecosystem shifts are to be expected as several aspects of climate change lead to differences in vegetation, hydrology and hydrologic connectivity. This alters the different effects and linkages of ecosystem drivers which finally transfers to the ecosystem services of alpine environments.

5.2 Bacterial spatio-temporal dynamic and possible future changes

The extensive sampling in the three catchments provided the general background of my thesis (chapter 1). I could show that the three sampled alpine floodplains harbor distinct bacterial communities. Large-scale ecological distances can influence community composition (Cho and Tiedje 2000, Whitaker *et al.,* 2003, Nemergut *et al.,* 2011). For instance, Reche *et al.* (2005) found distinctions between community composition in alpine lakes were related to distance. In concordance to my results, they also found no difference in numbers of operational taxonomic units detected. In this study, it seems that general physico-chemical characteristics that are related to geographical position dictated the community composition rather than isolation between locations. For example, I did not see a greater difference between the spatially more separated catchments Val Roseg and Loetschental, but rather differences between the Macun catchment and the other two. This was also true for the physico-chemical characteristics of the three catchments. Thus factors like altitude, vegetation, hydrologic characteristics (i.e. interconnected lakes, water availability) or geology (i.e. pH) seem to influence bacterial community composition at the large-scale and are themselves strongly correlated to physico-chemical characteristics of an ecosystem (Horner-Devine *et al.,* 2004, Fierer and Jackson 2006). As some bacteria show rapid proliferation, massive abundance, potentially high dispersal with little or no geographical barriers and are unlikely to go extinct, a potential seed bank may be available in any habitat (Roszak and Colwell 1987, Finlay and Clarke 1999, Curtis *et al.,* 2002, Fenchel and Finlay 2004, Martiny *et al.,* 2006). It has to be mentioned that the capacity of rapid (micro) evolution and horizontal gene transfer of bacteria makes it difficult to define so-called species. This may support the view of a potential seed bank to be defined by ecotypes or metacommunity lineages rather than by a binomial Linnaean species classification (Achtman and Wagner 2008). Regardless, it seems that environmental constraints determine bacterial community assemblages at the large scale as assessed by OTUs. Ecosystem functioning showed a similar trend with the Macun catchment being more distinct from the other two. This finding implies a linkage between function and structure of bacterial communities, which is one of the widely discussed topics in ecology (Nannipieri *et al.,* 2003, Hooper *et al.,* 2005, Gutknecht *et al.,* 2006, Cardinale *et al.,* 2009, Frossard *et al.,* 2012).

Water source was found to be an additional factor in forming community structure within the catchments. The few studies that have been conducted before within the same ecosystems could also find differences in cell abundance and specific communities inhabiting kryal and krenal stream sediments (Battin *et al.,* 2004, Logue *et al.,* 2004). Also, this separation seems to be linked strongly to the physico-chemical characteristics of the respective water source. Fierer *et al.* (2007a) reported within the relatively

small Hubbard Brook Forest watershed, a strong shift in bacterial community composition linked to stream water pH. I also found that functions were separated between water sources, except for Macun where no functional differentiation could be detected. Furthermore, there was a seasonal shift in community composition that was more pronounced in kryal systems, except for Macun where the krenal system showed a seasonal shift. Deviant from that, I could see a functional shift between sampling seasons in both water systems in Val Roseg and Loetschental. Again, the Macun catchment was temporally stable with regard to function. I could show that within the Val Roseg and Loetschental that functions were more directly linked to bacterial community composition in the kryal than in the krenal streams. Macun showed a linkage within the krenal systems. These results clearly show that the Macun catchment has not just a different bacterial community composition and functionality, but also different underlying mechanisms driving temporal variations. My finding of differences between community structure, functioning and their linkage at the smaller-scale of a catchment indicates different life strategies of stream sediment inhabiting bacteria within the different water systems. Krenal sediments seem to be more stable environments inhabited by generalists that show high functional plasticity (Battin *et al.,* 2004). In contrast, kryal sediments are habitats for specialists characterized by a coupled change in community structure and functioning. This change is in concert with shifting physico-chemical patterns and mechanical stress alterations during the different hydrological periods. Thus, seasonal changes in bacteria composition may reflect changes in metabolic properties. This can directly influence ecosystem functioning, as observed in glacial vs. non-glacial streams (Logue *et al.,* 2004).

In the second chapter, I investigated the importance of hydrologically-mediated physico-chemical and biotic connectivity between floodplain habitats in krenal systems. This represents the small-scale control mechanism on bacterial communities within this thesis. I could show that hydrology is a main driving factor for spatially mediated ecosystem functions. During periods of high hydrologic linkage, I detected directed gradual change in functionality along flow paths within the riparian zone as well as in the hyporheic sediments. During periods with low hydrologic linkage, there were just minor and undirected spatial patterns of bacterial functioning visible, thus floodplain ecosystem functions became more equal. The connectivity between in-stream and the riparian zone seemed to depend mainly on the landscape structuring; i.e., stream meanders led to an increased hydrological exchange with the riparian zone. It seems that these floodplains provide ecosystem functioning mediated by hydrology. For instance, during high hydrological connectivity nutrients are successively degraded along the flow path and there is a form of labor division.

Bacterial community composition was less affected by high hydrologic connectivity. This indicates a high degree of functional plasticity within the krenal systems. Although less influenced compared to functioning, bacterial community diversity and seasonality within the riparian zone seems to be partly maintained by seasonal changes in hydrology. During high connectivity in spring, bacterial communities could be formed due to an altered environment directly mediated by water physico-chemical characteristics or facilitated bacterial cell dispersal. During the low flow period in summer, isolation seems to drive communities but environmental forcing does not seem to separate riparian zone bacterial communities in a pronounced way as functions were mainly the same and bacterial communities did not show strong spatial patterns. When the riparian zone becomes hydrologically more active towards fall, bacterial communities face different environmental conditions as mirrored by the emerging spatial structuring of ecosystem functioning and bacterial communities became more diverse.

Mountain ecosystems around the world are being transformed at unprecedented rates. Many glaciers are expected to be gone by 2050 (IPCC 2007), and consequently the altitudinal distribution of terrestrial vegetation will change and thus induce an increase in organic matter resources (Hall and Fagre 2003). Autochthonous primary production can be large in alpine streams as they receive full sunlight (Jones 1995, Young 1999, Robinson *et al.,* 2003) and constitutes a main carbon input additionally to allochthonous particulate organic matter (Zah and Uehlinger 2001). Increased CO_2 concentrations may alter algal growth and additionally modifies carbon inputs into streams. Also, input of nutrients is likely to change with altered atmospheric inputs and changes in peak inputs due to, e.g., snow ablation (Brooks and Williams 1999, Burns 2003, Hiltbrunner *et al.,* 2005, Bowman *et al.,* 2006). Heterotrophic bacterial communities are strongly influenced by the nature of incoming organic material or the quality and quantity of nutrients (Sobczak and Findlay 2002, Findlay *et al.,* 2003, Judd *et al.,* 2006, Nelson 2009). Streams can show correlation in bacterial abundance and organic matter (Hall and Meyer 1998). Thus, these landscape changes will directly influence the physical, chemical, hydrological and, potentially, the metabolic (i.e. respiration) and functional properties as well as the functional and biological diversity of alpine aquatic ecosystems (Mulholland *et al.,* 1997, Hall and Meyer 1998). Landscape transformation also will directly affect water availability, hydrologic flow paths and linkages between terrestrial and aquatic ecosystems; a linkage clearly evident and important in alpine glacial floodplains (Malard *et al.,* 2006). Glaciers are an ecosystem heterogeneity agent. They seasonally feed alpine floodplains with kryal water, thus increasing temporal heterogeneity in physico-chemical characteristics and

providing ecosystem disturbance during high summer run-off. Microbial communities and metabolic activities can also be influenced by such altered flow velocities and turbulence (Cardinale *et al.,* 2002, Besemer *et al.,* 2007a, 2009b, 2009a).

Changes in bacteria composition and diversity, as a major response to environmental change, can have direct and cascading effects on ecosystem functioning. Research on biodiversity changes of microbial assemblages to environmental stress has increased in the last decade, recognizing that changes in biodiversity can threaten the capacity of ecosystems to deliver ecological and also human services (The State of the Nation's Ecosystems2008). I conducted the reciprocal transplant experiment to assess the impact of altered environmental factors as they can be expected in the future within these alpine floodplains (chapter 3). The manipulation of resource and nutritional state of sediments resulted in the loss of kryal landscape components or elevated organic matter input. I found that kryal and krenal bacterial community compositions were highly resistant to disturbance yet showed pronounced ecosystem function flexibility. The community structures showed different strengths in seasonal shifts and resistance to an altered water source. Krenal communities were generally more stable compared to the kryal one, although functional plasticity showed the same extent in both. Again these results indicate that krenal sediments are dominated by generalists, whereas kryal sediments are inhabited by specialists. I could not detect a strong and clear effect of nutrient amendments. This small impact may points out the highly complex but hierarchical structured interactions among the origin and seasonally shifted communities, water source and nutrients. The latter factor seems to be masked by the hierarchically superior ones. A shift from kryal to krenal water dominated systems may be functionally buffered by microbes. Although microbial functionality does not collapse and adapt to a new environment, such a shift will still have implications for carbon and nitrogen cycles at the ecosystem scale (Shen and He 2011). It is generally difficult to link specific processes to a specific microbial community assemblage (Allison and Martiny 2008). As my results show, there are different population strategies leading to adaptations to a new environment. Kryal sediments restructure their populations towards taxa performing better within a new physico-chemical environment. These taxa may represent the "occasional" species that emerge and may also show how seasonal fluctuations in kryal sediments may function. It is not clear at what diversity level ecosystem functions would be constrained. Even small numbers of observed taxa can show functional redundancy and increased functional abilities with higher diversity was found mainly in experiments using relatively small numbers of different taxa (Wohl *et al.,* 2004, Bell *et al.,* 2009, Langenheder *et al.,* 2010). Additionally, there are species-specific interactions determining the level of ecosystem functioning (Bell *et al.,* 2005).

Global change induced change in water sources in glaciated floodplains is expected to go along with widespread shifts in microbial communities, at least in the long term. A future loss in bacterial diversity could be expected when resident kryal specialists are unable to compete within an environment dominated and invaded by typical krenal species.

5.3 Strengths and limits of used field and methodological approaches and concepts

Every study trying to solve biological questions contains methodological as well as study design based limits in revealing answers. This is even truer for the latter if a study is conducted in the field. The choice of sites, spatial and temporal resolution and measured environmental variables are just a few critical components to name restricting the coverage of potential answers. This is also true for the present thesis.

The sampling effort from chapter one leads to results which can be generalized to some extent. This includes the linkage of physico-chemical factors to bacterial community structure and function. Regardless, generalized statements concluded from the results usually need categorization (i.e. different water systems). This surely is appropriate to cover the big picture and main mechanisms involved into forming bacterial communities and functions. The generalization sometimes does not work properly in explaining single case behavior. Community dynamic as seen in the Loetschental showed partial miss categorization (i.e. sites L1 and L9): community structures which were rather groundwater like but actually were sampled from a kryal stream and vice versa. Also physico-chemical changes along longitudinal flow paths are not taken into account when categorizing the water systems in a binary way. Temporal changes in physico-chemical characteristics can be apparent when, i.e., higher elevated ice shields or snow packs contribute to the krenal water budget in summer. Although the used statistical approaches are relative robust and with the binomial categorization interfering sites can be spotted easily, it may be of advantage to apply a more resolved categorization of water systems as the one used in the present thesis (Brown *et al.,* 2003, Brown *et al.,* 2009).

Correlation and causality is one of the open questions. Although a statistical correlation can be shown (i.e. between structure and physico-chemical milieu) and the used statistical models are based on methods aiming for parsimony, it is not clear if

one measured the causal environmental factors or just a thereto intercorrelated one. Additional laboratory experiments would be needed to ultimately show that a single factor causes a shift in community structure or function. Another dimension is for sure the general complexity of the environment. Many factors may have an influence on the same parameter (i.e. bacterial community composition) and potentially define its trajectory in time and space by highly complex interactions. The small impact of nutritional change in chapter three may be due to such interactions with other non-manipulated factors. The same is true for the results in chapter two. As direct hydrological connectivity and flow rates were not possible to assess in detail, I used a model based approach. Underlying assumptions fed to the models are for sure reasonable and the models can be qualitatively cross validated (i.e. an NMDS plot and explained variation along the flow path as assessed by AEM). Nevertheless, the lack of hydrological data (although water flows usually downhill) makes the numbers rather speculative yet outline trends giving insight into the importance of underlying mechanisms forming communities and present functions. Natural systems never appear in replicates (neither spatial nor temporal). They always represent a unique state when sampled and applied models helping interpreting the results have to be seen indicatory.

Besides the above mentioned hurdles, there is also the lack of a clear bacterial species concept. The herein used method for community fingerprinting (ARISA) for sure has its eligibility for assessing and comparing bacterial diversity. Regardless, it lacks the possibility to stick a bacterial “species” to a distinct function and thus can just be used to statistically assess general strength in linkage of structure and function. Also the measurement of potential enzymatic activities reveals the functional diversity on a relative coarse level. Enzymes catalyzing an equal biochemical reaction can be different in their amino acid composition and have, i.e., different performing optima under distinct environmental conditions (Braker *et al.,* 2000, Mienda and Yahya 2012). It might be that some specific functions are linked to community structure, whereas more broadly distributed functions are redundant within the microbial community. Thus, the actual functional diversity and potential (i.e. extend of functional redundancy) in a given habitat is not covered by the applied fluorometric assays. Ultimately, specific knowledge of microbial populations which perform specific processes is required to disentangle the relationship between microbial diversity and ecosystem functioning (Allison and Martiny 2008).

5.4 Concluding remarks, implications and outlook

A holistic view in the present thesis encompasses uncertainties in predicting future climate, difficulties in applying existing ecological models to the diverse microbial world and high temporal and spatial variability in the focal ecosystems. However, it is exactly this range in the template and range in response that allows disentangling the many factors ultimately influencing community assemblages. Also, the incorporation of algae and other species interactions should be pursued, as field biologists tend to focus on a specific set of organisms. Another challenge (mainly for sampling and experimental field campaigns) will be to see landscape structures such as lakes and streams in the context of their external environment. The hydrological interaction of in-stream and riparian zone microbial communities and their ecosystem functions as seen in chapter 2 clearly shows the importance of incorporating surrounding ecosystems into field studies addressing a specific structure in a landscape. Generally it seems that large-scale factors determine the active pool of microbes (catchment), whereas small scale differences in physico-chemical characteristics separate habitat patches (water systems). Within a habitat patch (sub-floodplain), communities show seasonal fluctuations in assemblages that are mediated by mechanisms linked to changes in hydrological connectivity or the physico-chemical habitat template. Potential trade-offs that bacterial species face seem to dictate in which habitat they can emerge.

Many new questions arose while working on the present thesis. Different experimental and study designs could be applied. A higher temporal resolution would give great insight into how and why communities turn over. The incorporation of additional factors, i.e. extreme rainfall events, should be taken into account. Future studies on stream bacterial populations in these glaciated Alpine systems should clearly start to incorporate potentially linked surrounding terrestrial areas. The same is true for the hydrology. The piezometer experiment clearly showed that hydrological peak periods influence both, structure and function, whereas different and rather stochastic mechanisms seem to work when the linkage becomes minor. Thus, the assessment on how landscape structures are linked should be higher weighted in studies investigating spatio-temporal dynamics of microbial populations. For instance, tracer experiments which are simultaneously performed when samples are taken could greatly improve the models used on hydrological connectivity. A more precise and quantitative assessment of flow path and flow rates would be a big step forward in the understanding of the importance of bacterial migration on the community structures and functions apparent in a habitat patch. Additional insight in the mechanisms leading to or maintaining a bacterial community in a habitat patch at a specific time would arise thereof. Future projects with similar set-up should put effort into a more precise hydrological assessment as well as a highly precise characterization of the habitat patch environment. Assessment of physico-chemical properties of a patch could helpful in separating the flow-mediated factors (i.e. dispersal and hydrological mediated physico-chemical factors) from strictly local factors. This could be

achieved with i.e. soil profiles taken next to the piezometers. Anyway, it still remains difficult or even impossible to assess rates of bacterial dispersal in a natural environment. Not to mention dispersal assessed on species level. Also the use of different techniques covering different ecosystem processes should be taken into account. I.e. nutrient uptake experiments would add a quantitative aspect of biogeochemical cycling. Such experiments may incorporate nutrient uptake within the riparian zone along a flow path to get a step further to a holistic understanding of how these ecosystems actually function.

High throughput sequencing covering functional and structural aspects could lead to a substantially better understanding of underlying mechanisms driving communities and ecosystem functions so they could be linked in a more straight-forward way. The sequencing of functional genes, their link to actual enzyme activities and the integration of proteomics and transcriptomics could largely contribute to unravel so far unanswered questions. In the near future, these techniques will become more accessible, affordable and the data volume dealable. Taken these molecular approaches together with field based data collection the general knowledge and the actual resolution of underlying mechanisms forming bacterial communities and functions within a complex environment should greatly improve in the near future.

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2008 **M. Sc. in Biology, Microbiology**, University of Zurich, Switzerland. Title of the thesis: Characterization of hybridizable and non-hybridizable DNA-containing particles in Lake Zurich. Advisor: Prof. Dr. Jakob Pernthaler, Department of Limnology, Institute of Plant Biology, University of Zurich, Switzerland.

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Working Experience

2007-2008 Cashier, East Cinemas AG, Zurich, Switzerland.

2003-2008 Assistant, Braune Roth AG, Engineering bureau for acoustic and building physics, Zurich, Switzerland.

1995-2000 Various part-time jobs: Vendor Coop Zumikon; Switzerland; Night watch in hospital, Zollikon, Switzerland; Factory worker OWO Zumikon, Switzerland.

Project Experience

2008-2010 Vice president, club “Battle for the Bracelet” poker tournament.

Publications

Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T. Spatio-temporal patterns and associated functions of lotic bacterial communities in three alpine floodplains.
(*Under review in the ISME Journal*)

Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T. Response of lotic microbial communities to altered water source and nutritional state in a glaciated alpine floodplain.
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Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T. Structure and function of bacterial communities within alpine floodplains: A hydrological point of view.
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Bodmer P., Döring M., Fumetti S. V., Freimann R, Christopher C. T. Respiration and microbial dynamics as indicators of floodplain heterogeneity: The alpine Urbach valley (Switzerland).
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Reviewing Experience

Freshwater Science
Limnology and Oceanography
Marine and Freshwater Research

Presentations at Conferences and Symposia

2012	(July) Association for the Sciences of Limnology and Oceanography (ASLO), Lake Biwa, Otsu, Japan, Oral presentation: *Functioning of lotic bacterial communities in alpine floodplains*. Robinson. C. T., Bürgmann H., Findlay S. E. G., Freimann R.
2011	(June) Symposium for European Freshwater Sciences (SEFS), Annual meeting: Freshwater ecosystems-challenges between conservation and management under global change, Girona, Spain, Invited oral presentation: *Spatial-temporal patterns and shifting role of bacteria in ecosystem functioning in Swiss alpine floodplains*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2011	(May) North American Benthological Society (NABS), Annual meeting: Responding to the global water crisis, Providence, Rhode Island, USA, Invited oral presentation: *Reciprocal transplant experiments for examining microbial community assembly in alpine floodplains*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2011	(May) 1th Eco Symposium, Zurich, Switzerland, Oral presentation: *Spatio-temporal patterns and shifting role of bacteria in ecosystem functioning in Swiss alpine floodplains*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2011	(February) 4th Swiss Microbial Ecology Meeting (SME), Engelberg, Switzerland, Oral presentation: *Reciprocal transplant experiments for examining microbial community assembly in alpine floodplains*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2010	(July) 10th Anniversary Macun (Swiss National Park), Lavin, Switzerland, Oral presentation: *Macun as an example of microbial diversity and functions in alpine floodplains*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2010	(June) ASLO-NABS joint Meeting, Aquatic sciences: Global changes from the center to the edge, Santa Fe, NM, USA, Poster presentation: *Microbial biodiversity in Swiss alpine floodplains: Seasonal aspects and the shifting role of bacteria in ecosystem functioning*. Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.
2009	(April) Annual Meeting Swiss National Park (SNP), Zernez, Switzerland, Oral presentation: *Terrestrial / aquatic linkages in microbial biodiversity in alpine floodplains: Shifting role of bacteria in ecosystem functioning* (MICROLINK). Freimann R., Bürgmann H., Findlay S. E. G., Robinson. C. T.

Technical Skills

Microscopy:	Light microscopy, epifluorescence microscopy (EF), confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), transmissions electron microscopy (TEM), atomic force microscopy (AFM).
Microbiology:	CARD-FISH, nucleic acid extraction, PCR, ARISA, DGGE, immunoassays, proteomics, HPLC, MS, enzyme activity analysis, cell abundance, flow-cytometry (FACS), culture based methods.
Field:	Nutrient uptake, ecosystem respiration and production, piezometer applications, general geographical-, limnological- and geochemical methodologies.
Software:	General (MS Office applications, Adobe PhotoShop), bioinformatics and microscopy (Gene mapper, ImageJ, CellC, FlowJo, Imaris, several device based software), statistics and modeling (R, SPSS, Sigma Plot), essential knowledge in multivariate statistics and ecosystem/spatial modeling (R).

Advanced Training

2009	Course “Fundamentals of Ecosystem Ecology”, Cary Institute of Ecosystem Studies, Millbrook NY, USA.