1 **A decade of irrigation transforms the soil microbiome of a semi-arid pine forest**

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Abstract

The impact of climate change on the soil microbiome potentially alters the biogeochemical cycle of terrestrial ecosystems. In semi-arid environments, water availability is a major constraint on biogeochemical cycles due to the combination of high summer temperatures and low rainfall. Here, we explored how ten years of irrigation of a water-limited pine forest in the central European Alps altered the soil microbiome and associated ecosystem functioning. A decade of irrigation stimulated tree growth, resulting in higher crown cover, larger yearly increments of tree biomass, increased litter fall, and greater root biomass. Greater amounts of plant-derived inputs associated with increased primary production in the irrigated forest stands stimulated soil microbial activity coupled to pronounced shifts in the microbiome from largely oligotrophic to more copiotrophic lifestyles. Microbial groups benefitting from increased resource availabilities (litter, rhizodeposits) thrived under irrigation, leading to enhanced soil organic matter mineralization and carbon respired from irrigated soils. This unique long-term study provides new insights into the impact of precipitation changes on the soil microbiome and associated ecosystem functioning in a water-limited pine forest ecosystem and improves our understanding of the persistency of long-term soil carbon stocks in a changing climate.

36 Introduction

37 Climate scenarios for the late twenty-first century forecast continued global warming and an
 38 increased frequency and intensity of extreme climatic events (Seneviratne *et al.* 2012; Zhao &
 39 Running 2010). Such forces will affect terrestrial ecosystems worldwide, potentially resulting in a
 40 reduction of the global net primary production and negative biogeochemical feedbacks to the climate
 41 (Reichstein *et al.* 2013). Shifts in rainfall patterns and increasing temperatures will likely cause tree
 42 mortality (Anderegg *et al.* 2013; Rebetez & Dobbertin 2004). Climate extremes induce a series of
 43 interconnected effects that act synergistically at the organism, ecosystem and regional scale, and
 44 thereby have the potential to profoundly alter the carbon balance of semi-arid ecosystems with direct
 45 feedback to the climate system (Poulter *et al.* 2014).

46 The complex response of the soil system and its indigenous microbiome to precipitation change is
 47 pivotal but still poorly understood (de Vries *et al.* 2012). Water availability may impact the soil
 48 microbiome directly (Manzoni *et al.* 2012), as well as indirectly through changes in vegetation or
 49 substrate supply (Nielsen & Ball 2015). While many studies have been conducted to determine how
 50 plant communities in dryland ecosystems respond to precipitation change, research about the impacts
 51 of altered precipitation on the soil microbiome are rare (Evans & Wallenstein 2014). In particular, little
 52 is known about the effect of longer term changes in soil water availability on the soil microbial
 53 communities and associated ecosystem functions. Leutzinger *et al.* (2011) pointed out that long-term
 54 manipulation experiments are urgently needed in climate impact research, in particular in forests. The
 55 temporal scale plays an important role in predicting ecosystem responses (e.g. above- and
 56 belowground biomass, litter fall, soil C content) to precipitation change, whereas short-term
 57 experiments might fail to capture some of the processes and feedback mechanisms that occur over
 58 longer time scales. Most studies in forests have focused on rainfall exclusion approaches (Bouskill *et al.*
 59 *et al.* 2013), and only few studies have examined changes in microbial community composition
 60 following rainfall addition (Brzostek *et al.* 2012; Cregger *et al.* 2012). However, rainfall addition
 61 experiments help to determine, if systems are water-limited, which in turn is critical for gaining more
 62 complete insights into the biotic responses to long-term impacts of climate change.

Here, we examined the response of the soil microbiome and associated ecosystem functions to precipitation changes using a unique long-term irrigation experiment in a water-limited pine forest ecosystem in the central European Alps (Dobbertin *et al.* 2010). The irrigation experiment was established in the Pfyn forest in 2003 to better understand how forest ecosystems respond to water limitation (Dobbertin *et al.* 2010). The Pfyn forest represents the largest continuous forest of Scots pine (*Pinus sylvestris*) in Switzerland and is located in the dry Rhone Valley. Large-scale Scots pine forests in the transition zone between continental and Mediterranean climates are characteristic landscape elements in dry and warm inner-alpine valleys of the Central Alps (Rigling *et al.* 2013). Increasing Scots pine mortality has been noted for several decades, with a dieback of up to 50% in water-limited stands in the Swiss Rhone Valley since 1995 as well as in other valleys of the central Alps in Italy and Austria (Rebetez & Dobbertin 2004; Vacchiano *et al.* 2012). Although average annual precipitation has remained constant over the last decades, there is evidence that climate warming has increased evaporation rates, and that water has become the main factor limiting growth and reducing stress resilience of trees (Rigling *et al.*, 2013). It was therefore hypothesized that reducing stress from water limitation via irrigation would improve tree vitality and reduce mortality. Indeed, after 3 to 9 years of irrigation, trees showed increased leaf area (Dobbertin *et al.* 2010) and greater fine root biomass (Herzog *et al.* 2014). Furthermore, irrigation caused significant shifts in plant community composition and increased vegetation cover (Herzog *et al.* 2014). In response to the doubling of precipitation amount (from 520 yr⁻¹ to about 1100 mm yr⁻¹) over the decade-long experimental period, the monthly mean volumetric water content in the top soil (down to 10 cm depth) increased significantly ($P = 0.022$) from 27.5% in the control plots to 34.1% in the irrigated plots (Table 1; Supplementary Fig. 1).

To the best of our knowledge, this is the first long-term (≥ 10 years) rainfall addition experiment to address the effect of precipitation change on the soil microbiome and associated ecosystem functions, especially in an ecosystem with a strong history of water limitation. We tested if and how precipitation change alters bacterial and fungal diversity in the soil surface and whether the responses to irrigation penetrate in the mineral sub-surface soil horizons. We hypothesized that increased primary production and associated changes in resource availabilities (litter, rhizodeposits) after a decade of irrigation

introduce significant shifts to the forest soil microbiome that are in agreement with the current understanding of oligotrophic and copiotrophic life strategies (Fierer *et al.* 2007). We hypothesized that increased litter fall and stimulated root growth under irrigation promote the occurrence of copiotrophs such as Proteobacteria and Bacteroidetes (Fierer *et al.* 2007), casual mesotrophic root invaders such as zygomycete fungi (Dix & Webster 2012; Richardson 2009), and early litter saprobes such as Ascomycota (Voriskova & Baldrian 2013), whereas water-limited conditions favor oligotrophic, metabolically versatile, or drought-tolerant bacteria such as Acidobacteria, Actinobacteria, Gemmatimonadetes (DeBruyn *et al.* 2011; Fierer *et al.* 2007; Hanada & Sekiguchi 2014; Kielak *et al.* 2016; Mohammadipanah & Wink 2015; Rosenberg *et al.* 2014) as well as ectomycorrhizal fungi such as Basidiomycota as a consequence of a shift in vegetation (Herzog *et al.* 2014) and increased symbiosis to protect the plants from desiccation and starvation (Brunner *et al.* 2015). Changes in quantity and/or quality of C inputs and shifts in bacterial and fungal community structures following long-term irrigation of dry soils might in turn alter soil ecosystem functioning (SOM mineralization and transformation) with important implications for the fate of long-term soil C stocks under climate change.

Material and methods

Experimental set up and soil sampling

The Pfyn forest long-term irrigation experiment is situated in the Rhone Valley of Switzerland (46°18'N, 7°37'E, 615 m.a.s.l.), in a Scots pine (*Pinus sylvestris*) forest with occasional interspersed pubescent oak (*Quercus pubescens*). The study area, including 899 numbered and geo-referenced pine trees, was divided into eight plots of 25 x 40 m (1000 m²) with 5 m buffer areas between and around each plot (Dobbertin *et al.* 2010; Herzog *et al.* 2014). The plots were aligned side by side along a channel fed by the Rhone River, from where water was taken to irrigate four randomly selected plots (further termed “irrigated”). Four plots were left untreated (further termed “dry”). Irrigation was started for the first time in spring 2003. The irrigation system was activated on rainless nights during the vegetation period (May-October), doubling the annual rainfall amount (Feichtinger *et al.* 2014).

Soils were sampled with a quantitative soil pit approach using a metal frame of 20 x 20 cm in October 2012 at the end of the irrigation period. Four replicate samples were collected at four different locations in each of the eight plots (at least 5 m distance from the plot edge) at three soil depths, i.e. the organic F-Horizon (Org) as well as at 0-2 cm depth (Min2) and 5-10 cm depth (Min10) of the mineral soil. The four individual replicates per plot and depth were pooled for each study plot separately, leaving the independent plots as the level of replication. This gave a total of 24 soil samples (2 treatments × 4 independent plots × 3 soil horizons). The fresh soil samples were homogenized using a 2 mm sieve. For estimation of soil microbial biomass and carbon mineralization, soils were processed directly. For PLFA and DNA analysis, soils were immediately frozen and kept at - 80°C. For soil analysis, subsamples were dried at 60°C and ground with a ball mill.

Basic soil and plant characteristics

Volumetric soil water content was monitored hourly using time domain reflectometry (Tektronix 1502B cable tester, Beaverton, OR) at 10 cm soil depth at four different locations in irrigated and dry plots. The mean volumetric water content in the soil significantly increased from 27.8% in the dry plots to 34.3% in the irrigated plot (Herzog *et al.* 2014; Supplementary Figure S1). Soil pH was determined in 0.01 M CaCl₂. Total carbon and nitrogen content were measured using an automated elemental analyser (Euro EA 3000, HEKAtech, Germany). Soil organic C (SOC) stock was determined by multiplying SOC concentrations with the bulk densities and the volume of the corresponding soil layers considering the stone contents.

Fine root sampling was executed twice, i.e. in May 2003 and in May 2012 each time before the irrigation started. The fine roots were sampled by sampling four soil cores at a distance of 1 m from each of three trees per plot with soil coring cylinder (Ø 45 mm) and analyzed according to Herzog *et al.* (2014). Cellulose extraction from fine roots and ¹³C measurements of the extracted cellulose was performed following the protocol of Herzog *et al.* (2014).

Litter fall was collected by five litter-traps of 50 x 50 cm placed on the ground in each of the eight plots (total n=40). The litter material was sampled in autumn and spring, and dried at 60°C for two days before weighing. The above-ground tree biomass was estimated by using allometric relationships

(stem diameter, tree height; Etzold *et al.* 2014) and annual increments of the above-ground volume of all individual trees were compared with the tree volume of 2002. Crown cover was recorded in 2008 according to Dobbertin *et al.* (2010). Plant richness was recorded in May 2012 and estimated according to Herzog *et al.* (2014).

Microbial biomass carbon, carbon mineralization and respiration measurements

Phospholipid fatty acids (PLFA) were extracted and measured using gas chromatography–mass spectrometry (GC-MS) as described previously (Frey *et al.* 2006; Hagedorn *et al.* 2013). The fatty acids 18:2 ω 6,9 and 18:1 ω 9 were used as a biomarker for fungi (Bååth & Anderson 2003), while the fatty acids 16:1 ω 7, cy17:0, 18:1 ω 7, cy19:0, i15:0, a15:0, i16:0, i17:0, a17:0 were markers for bacteria (Zogg *et al.* 1997).

Soil respiration was measured in the field using an infrared gas analyser (IRGA) on a monthly basis in 2012 according to Guelland *et al.* (2013). Soil respiration is directly measured in the field and serves as the total CO₂ emissions from soils including heterotrophic and autotrophic respiration. In this system, it can be expected that a significant amount of the respired CO₂ is actually coming from the roots of the trees and not from the soil microbiota. Basal respiration was determined under field moist conditions (i.e. different for dry and irrigated soils) in a closed loop system with a CO₂ analyzer (LI-COR-840) at 20°C. Potential C mineralization was measured under standardized moisture conditions (adjusted to 50% water holding capacity) on a weekly basis using the same method as described above.

Pyrosequencing of bacterial and fungal ribosomal markers

Nucleic acids were extracted from 0.5 g soils using a bead-beating procedure as described previously (Frey *et al.* 2008). DNA concentrations were determined using PicoGreen (Molecular Probes, Eugene, OR, USA). PCR amplification of partial bacterial small-subunit ribosomal RNA genes (region V1–V3 of 16S rRNA) and fungal ribosomal internal transcribed spacer region ITS2 was performed using 20 ng of soil DNA, as described previously (Hartmann *et al.* 2012). Purified amplicons with different barcoded primers were pooled in equimolar concentrations and sequenced

using the GS-FLX Titanium technology (Roche 454 Life Sciences, Branford, CT, USA) at the Genome Quebec Innovation Center, Montreal, Canada.

Quality control, clustering into operational taxonomic units (OTUs), and taxonomic classification of bacterial and fungal reads was performed according to Hartmann *et al.* (2014). The 454 pyrosequencing reads were deposited in the European Nucleotide Archive under the accession number PRJEB14824. A FASTA-formatted file containing all high quality sequences analyzed in this study is provided as Supplementary Data 1.

Statistical analyses of microbial data

Alpha-diversity estimates included the Shannon diversity index based on iteratively rarified (at 3270 bacterial and 2312 fungal reads) OTU abundance data calculated in MOTHUR (Schloss *et al.* 2009) as well as rarefaction interpolation and extrapolation analysis of the observed richness (Chao *et al.* 2014) using the package iNEXT (Hsieh *et al.* 2013) in R (R Core Team 2014). Beta-diversity was measured by Bray-Curtis similarities calculated from standardized square-root transformed OTU abundances. Square-root transformation of standardized data, also known as Hellinger transformation, is used to downweight the contributions of quantitatively dominant taxa to the similarities calculated between samples without losing the influence of less abundant taxa (Clarke & Warwick 2001). Differences in β -diversity across samples were examined by principal coordinate analysis (PCO) using the *cmdscale* function in R. Bacterial and fungal PCO ordinations and underlying Bray-Curtis resemblance matrices were compared using Procrustes analysis and Mantel testing, respectively, as implemented in the R package *vegan* (Oksanen *et al.* 2016). Irrigation effects along the soil depth profile on α - and β -diversity were quantified using univariate and multivariate permutational analysis of variance (PERMANOVA, Anderson 2001), respectively. Homogeneity of variances across treatments and soil horizons were examined using permutational analysis of multivariate dispersions (PERMDISP, Anderson 2006). PERMANOVA and PERMDISP were performed using the homonymous routines implemented in PRIMER6+ (Clarke & Gorley 2006). Taxon-level responses to irrigation were assessed at different taxonomic levels from phylum to OTU using univariate PERMANOVA as implemented by the *adonis* function in the R package *vegan*. In order to retrieve

taxa showing a universal response to irrigation or long-term water limitation, relative abundance data were centered by depth and scaled (combined also known as z-transformation) prior to PERMANOVA and visualization. Adjustments for multiple hypothesis testing for this type of data is potentially problematic and lead to an inflation of false negatives, since the basic assumption of uniform p-value distribution under the null hypothesis is violated for sparse count data, resulting in a bimodal p-value distribution (Storey & Tibshirani 2003). We performed multiple testing adjustment using the false discovery rate correction according to Storey & Tibshirani (2003) implemented in the R package qvalue (Storey *et al.* 2015), but provide the exact p-value and the adjusted q-value alongside for all tests. Hierarchical taxonomic networks were generated based on the taxonomic path of each OTU and the OpenCL-accelerated Allegro Fruchterman–Reingold algorithm in CYTOSCAPE 3.3 (Shannon *et al.* 2003) as described previously (Frey *et al.* 2016). Information on treatment-sensitive OTUs were subsequently mapped onto these networks.

Results and Discussion

Primary production and associated changes in microbial diversity

A decade of irrigation stimulated primary production, resulting in higher crown cover, larger yearly increments in tree biomass (approximately 2% of Scots pine biomass), greater root biomass, and increased litter production (Table 1). A shift to more depleted ^{13}C in the fine root C further demonstrates an increased photosynthetic activity of Scots pine under irrigation due to increased discrimination against the ^{13}C isotope (Table 1). Increased litter fall and rhizodeposition (approximated by root growth and ^{13}C), but also shifts in vegetation with a greater coverage of deciduous shrubs and an overall shift from pubescent oak to Scots pine in irrigated plots from (Herzog *et al.* 2014), have likely changed the quantity and quality of C input into the soil and, thus, might have increased soil C availability under irrigation with direct consequences for the soil microbiome (Gschwendtner *et al.* 2015; Pascault *et al.* 2013). Stimulation of primary production with irrigation was also evidenced by a stimulated soil respiration (i.e. total autotrophic and heterotrophic respiration in the field including plant and microbial activity) and basal respiration (microbial respiration under field moist conditions), suggesting that the greater amount of C input in irrigated soils stimulated

microbial activity (Table 1). Stimulated microbial activity in irrigated soils, however, was not associated with an increase in microbial biomass (DNA or PLFA approximations) (Table 1). Also, no shifts in the fungal-to-bacterial biomass ratio was observed (Table 1), which was surprising as fungi are proposedly more resistant to water limitation than bacteria (de Vries *et al.* 2012), theoretically resulting in a lower fungal-to-bacterial biomass ratio under irrigation.

We analyzed the effects of long-term irrigation on soil microbial diversity at different soil depths by 454 pyrosequencing of ribosomal marker genes. A total of 128,245 (5344 ± 1081 per sample) bacterial 16S_{V1-V2} and 124,407 (5184 ± 1434) fungal ITS2 high-quality sequences representing 3871 (776 ± 121 per sample) bacterial and 1512 (238 ± 69) fungal OTUs were obtained for the 24 soil samples. Long-term irrigation significantly altered microbial β -diversity and both bacteria and fungi responded similarly (Figure 1, Table 2). The communities were spatially structured along the soil depth gradient, but the irrigation effect was not depth-dependent. Irrigation effects on microbial α -diversity were more subtle. Both bacterial and fungal Shannon indices did not show any significant differences between treatments and soil depths, although bacterial diversity tended ($P = 0.079$) to increase with irrigation (Figure 1, Table 2). Examining only the mineral horizons gave a slightly more robust trend of increased bacterial ($P=0.60$) and fungal ($P=0.51$) diversity under irrigation (Table 2). It has previously been hypothesized that input of fresh organic matter stimulates copiotrophic organisms coupled to an increased production of extracellular enzymes that ultimately also attack the more recalcitrant soil organic matter pool, which in turn stimulates organisms able to degrade more complex compounds and altogether increases microbial diversity (Pascault *et al.* 2013). Examination of α -diversity at greater sequencing depths using rarefaction and extrapolation analysis of the observed richness supported the trend to slightly increased bacterial α -diversity under irrigation, whereas fungal α -diversity tended to be higher under water limitation in the organic horizon and higher under irrigation in the mineral horizon (Figure S2).

We further explored which taxa are responsible for the observed shifts in β -diversity at various taxonomic levels from phylum to OTU. These analyses enabled us to find microbial taxa showing a universal response to irrigation or water limitation in order to (1) identify potential ecological adaptation mechanisms to long-term arid conditions as well as to (2) evaluate whether a decade of

irrigation favored microbial life strategies that are typical for responses to changed resource availabilities under increased primary production (Fierer *et al.* 2007; Goldfarb *et al.* 2011). The community was dominated by taxonomic groups commonly observed in forest soils including Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes, as well as Basidiomycota, Ascomycota, and Zygomycota (Figure 2). Treatment-sensitive OTUs were broadly distributed across the taxonomic hierarchy and revealed substantial response heterogeneity within the individual phyla; however, some phyla such as Proteobacteria, Actinobacteria, and Acidobacteria showed a clear accumulation of sensitive OTUs (Figure 2). Qualitatively, Proteobacteria showed an accumulation of OTUs responding positively to irrigation, whereas Actinobacteria had a higher number of OTUs adapted to water limitation. Acidobacteria, on the other hand, showed a very heterogeneous picture with similar number of OTUs associated with the two states. In the following, we aim at characterizing the most salient shifts; however, we provide the complete test statistics of all taxa both at OTU level (Supplementary Data 2) as well as at various taxonomic levels from phylum to genus (Supplementary Figures S4 and S5), alongside a more detailed discussion about sensitive taxa (Supplementary Results).

Long-term irrigation induced shifts from oligotrophic to copiotrophic lifestyles

Irrigation led to consistent phylum-level changes in soil bacterial and fungal communities (Figure 3). Proteobacteria, Zygomycota, and Planctomycetes as well as some less abundant candidate phyla including WS3, GN04, and OP11 increased under irrigation. In contrast, Gemmatimonadetes, Actinobacteria, Armatimonadetes, and Acidobacteria were more abundant in the dry plots. As outlined in the following sections, the copiotroph–oligotroph trade-off also known as r- and K-selection theory (Fierer *et al.*, 2007) might explain some of these results. This hypothesis predicts that largely copiotrophic organisms (e.g. Proteobacteria, Zygomycota) thrive in soils with higher net carbon mineralization rate, whereas oligotrophic groups (e.g. Acidobacteria) dominate in soils of low carbon availability (Fierer *et al.* 2007; Fierer *et al.* 2012; Fontaine *et al.* 2003). In the present study, increased carbon availability via stimulated net primary productivity under irrigation appeared to favor copiotrophic over oligotrophic lifestyles. Since it can be expected that copiotrophic taxa have higher

rates of metabolic activity per unit biomass, higher turnover rates, and higher substrate use efficiencies yielding a smaller standing biomass pool (Fierer *et al.* 2012), such a shift from oligotrophic to copiotrophic lifestyle would also explain the unaltered microbial biomass despite the higher carbon mineralization rate observed in the irrigated stands (Table 1). We conclude that differences in basal respiration between dry and irrigated soils largely represent changes in microbial activities based on the different soil moisture levels, whereas differences in potential C mineralization show either altered carbon substrate availabilities or shifts in microbial community structure. Therefore, microbial activity is not only enhanced under irrigation because of increased soil moisture, but also because of an altered carbon use efficiency of the soil microbiome. In the following, we will discuss the response of the individual phyla in the context of what is known about their lifestyle from the literature.

Proteobacteria represented the most abundant bacterial phylum and revealed the strongest increase in relative abundance under irrigation (Figure 3). This is in agreement with their postulated copiotrophic lifestyle and prevalence under increased resource availability (Fierer *et al.* 2007; Koyama *et al.* 2014; Zeng *et al.* 2016). Members of the Proteobacteria are physiologically and ecologically extremely diverse, with lifestyles ranging from key players in carbon, nitrogen and sulfur cycling to symbiotic and parasitic organisms (Kersters *et al.* 2001), making it very difficult to predict their overall behavior under water limitation. However, Proteobacteria have been shown to positively correlate with soil moisture and precipitation (Evans *et al.* 2014; Zeng *et al.* 2016), to be sensitive to short-term drought scenarios (Bouskill *et al.* 2013; Chodak *et al.* 2015), and to consistently decrease during long-term water limitation in a Mediterranean forest (Curiel Yuste *et al.* 2014), ultimately supporting what has been observed in the present study. The positive proteobacterial response to irrigation was largely attributed to the Betaproteobacteria and, to some degree, Gammaproteobacteria (Figure 3), which have been identified as the proteobacterial members with typical copiotrophic characteristics (Fierer *et al.* 2007); however, significant shifts at lower taxonomic levels also took place in the other classes (see Supplementary Results).

The polyphyletic fungal group formerly known as Zygomycota (Hibbett *et al.* 2007) and more recently been split up into the phyla Mucoromycota and Zoopagomycota (Spatafora *et al.* 2016) also increased under irrigation (Figure 3). Many zygomycetes thrive as ubiquitous saprobes in soil and

litter (Richardson 2009), but there has yet been little effort to evaluate their response to water limitation and irrigation. The observed increase of zygomycetes under irrigation was largely driven by the genera *Mortierella*, *Umbelopsis* and *Zygorhynchus* (all genera within the Mucoromycota), which are among the most common genera found in forest soils and play a major role in C-cycling as efficient decomposers particularly during the first stage of decay when labile carbohydrates are readily available, although some species are also able to degrade more complex substances like chitin and hemicellulose (Dix & Webster 2012; Lee Taylor & Sinsabaugh 2015). These taxa are also among the first organisms to colonize plant roots and thrive rapidly on easily available C sources such as monosaccharides from root exudations until being replaced by a more stable fungal community at later stages of the root development (Dix & Webster 2012). The mesotrophic and fast-growing features of the zygomycete fungi (Richardson 2009) allowed them to thrive under increased input of fresh C-sources (i.e. root exudation, litter fall) under irrigation.

Planctomycetes also tended to be more abundant under irrigation (Figure 3), but the literature provides equivocal results regarding their response to precipitation change (Bachar *et al.* 2010; Barnard *et al.* 2013; Bouskill *et al.* 2013; von Rein *et al.* 2016; Waring & Hawkes 2015; Zeng *et al.* 2016), likely depending on what subgroups are detected. Planctomycetes are usually slow growing, aerobic bacteria that have several unusual cell characteristics (Fuerst & Sagulenko 2011). The lack of cultured representatives still limits our understanding of these bacteria, making it difficult to speculate about potential adaptation mechanisms under water limitation and long-term irrigation; however, it has been suggested some members can grow on complex heteropolysaccharides such as from decaying wood (Wang *et al.* 2015), which could be a reason for their prevalence under increased primary production in the irrigated plots (Supplementary Results).

We also still know very little about the candidate phyla WS3, GN04, and OP11 that have increased under irrigation. Members of these uncultured groups of bacteria have only recently been characterized in more detail and feature small streamlined genomes with limited metabolic capabilities and potentially symbiotic lifestyles (Brown *et al.* 2015; Youssef *et al.* 2015).

Other groups such as Actinobacteria, Gemmatimonadetes, Acidobacteria, and Armatimonadetes were more abundant in the water-limited forest plots (Figure 3). Under long-term water limitation,

factors such as metabolic versatility to degrade complex organic compounds, higher C-use efficiency, and tolerance towards desiccation and nutrient limitations through cellular modifications (osmotic protectants, dormancy) are important traits for survival and growth (Schimel *et al.* 2007). Actinobacteria, for example, are ubiquitous and frequently saprophytic organisms able to degrade recalcitrant carbon, play a vital role in the soil C-cycle, and have been shown to be highly resistant towards desiccation and C starvation (Bull 2011; Mohammadipanah & Wink 2015; Rosenberg *et al.* 2014; Ventura *et al.* 2007). The ability to decompose recalcitrant compounds is an important trait in arid systems when more readily available substrates are rather limited. Furthermore, many members of the Actinobacteria are capable of spore formation and filamentous growth, which facilitates survival under conditions of low hydraulic connectivity in unsaturated soils (Wolf *et al.* 2014). With these capabilities, Actinobacteria are known to compete well under arid conditions, correlate negatively with soil moisture (Zeng *et al.* 2016), and appear to be adapted to semi-arid soil environments (Banerjee *et al.* 2016; Barnard *et al.* 2013; Bouskill *et al.* 2013; Chodak *et al.* 2015; Curiel Yuste *et al.* 2014; Felsmann *et al.* 2015; von Rein *et al.* 2016; Waring & Hawkes 2015), supporting our observations (Figure 3).

Gemmatimonadetes are abundant but physiologically poorly studied soil bacteria, and biogeographic surveys suggest a strong adaption to environments of low moisture contents (DeBruyn *et al.* 2011). The presence of Gemmatimonadetes in arid and often extremely nutrient-limited environments such as cave walls (Pašić *et al.* 2010; Zhou *et al.* 2007), weathering rocks (Cockell *et al.* 2009), or subglacial sediments (Rime *et al.* 2015; Rime *et al.* 2016) indicates a strong tolerance towards desiccation and adaptation to oligotrophic conditions (Hanada & Sekiguchi 2014), which is again in agreement with our hypothesized framework. However, the impact of water availability on the abundance and distribution of Gemmatimonadetes is not yet understood as previous studies have found both positive (Curiel Yuste *et al.* 2014; DeBruyn *et al.* 2011) and negative (Chodak *et al.* 2015; Zeng *et al.* 2016) relationships with water limitation.

Acidobacteria are ubiquitous, diverse, desiccation tolerant, and largely oligotrophic bacteria adapted to nutrient-limited environments (Kielak *et al.* 2016), and play an important role in C-cycling (Lladó *et al.* 2016). Soil Acidobacteria have been shown to be abundant under low resource

availability (Fierer *et al.* 2007; Koyama *et al.* 2014) and to increase during long-term water limitation in a Mediterranean forest (Curiel Yuste *et al.* 2014), supporting our observations (Figure 3).

Armatimonadetes is a more recently discovered, moderately abundant but phylogenetically diverse bacterial phylum with very few cultured representatives (Lee *et al.* 2014). Few common phenotypic characteristics have so far emerged, but all cultivated members share an aerobic oligotrophic metabolism that, again, is in agreement with the copiotroph-oligotroph framework under water limitation and irrigation. In agreement with our observations (Figure 3), Armatimonadetes were negatively correlated with soil moisture and mean annual precipitation along a latitudinal gradient in China (Zeng *et al.* 2016). Taken together, Actinobacteria, Gemmatimonadetes, Acidobacteria, and Armatimonadetes appear to be well adapted to semi-arid soil ecosystems and potentially get outcompeted under increased primary production after long-term irrigation.

Other bacterial phyla commonly found in soil such as Chloroflexi (4.0%), Firmicutes (0.3%) and Verrucomicrobia (0.6%) showed no consistent responses to irrigation (Figure 3). Previous studies also reported rather equivocal results of these groups in response to water limitation and precipitation. For example, some studies suggested that Verrucomicrobia follow a more copiotrophic lifestyle with preference for moist soils (Barnard *et al.* 2013; Chodak *et al.* 2015; Curiel Yuste *et al.* 2014), whereas others have suggested a more oligotrophic life-strategy of Verrucomicrobia in respect to water availability (Bachar *et al.* 2010; von Rein *et al.* 2016). Similar reports have been found for Firmicutes, documenting negative (Bouskill *et al.* 2013), positive (Bachar *et al.* 2010; Li *et al.* 2016) or no association (Barnard *et al.* 2013; Curiel Yuste *et al.* 2014) with arid conditions, such that it remains challenging to classify them according to the copiotroph-oligotroph hypothesis (Fierer *et al.* 2007). A more resistant life strategy in relation to water limitation and irrigation was also reported for the phylum Chloroflexi (Bachar *et al.* 2010; Barnard *et al.* 2013; Zeng *et al.* 2016). However, these equivocal results also suggest that surveying phylum-level abundances is a simplistic view that ignores potentially heterogeneous responses at lower taxonomic levels (see discussion below).

Bacteroidetes is another bacterial phylum commonly considered to feature copiotrophic lifestyles (Fierer *et al.* 2007), usually well represented in well-watered soils, and reported to decrease in numbers under drought (Chodak *et al.* 2015; Curiel Yuste *et al.* 2014). However, this phylum did not

respond to irrigation when all soil horizons were included in the analysis (Figure 3). Since inter-sample dispersion was highest in the organic horizon and decreased with depth (data not shown), some underlying shifts that were confined to the mineral horizon were masked and became evident when excluding the organic layer from the analysis (Figure S3). Whereas all above observations also hold true when only considering the mineral soil, the phylum Bacteroidetes revealed a strong increase under irrigation in the mineral soil, further confirming the copiotroph-oligotroph hypothesis. It was also notable that effects at class level, for example for Beta-, Gamma-, and Deltaproteobacteria became more pronounced when considering the mineral soil only (Figure S3). At this point, it is also noteworthy to mention that certain microbial populations could be brought into the soil through the irrigation system that was fed with water from the Rhone River. However, it is unlikely that microbial populations adapted to aquatic systems and inoculated at a relatively low abundance will establish and thrive in forest soils such that they override the indigenous community to a degree that such strong treatment effects are observed.

Response of fungal decomposers and symbionts to irrigation

We hypothesized that increased litter fall under irrigation promotes the occurrence of saprobic fungi (predominantly Ascomycota), whereas water limitation favors ectomycorrhizal relationships (predominantly Basidiomycota). Among the decomposers, it has been suggested that rhizomorph and cord-forming Basidiomycota rapidly colonize fresh litter under moist conditions, but that drought-tolerant dark-septate Ascomycota predominate over Basidiomycota as both plant symbionts and decomposers in arid environments (Lee Taylor & Sinsabaugh 2015). In the present study, Ascomycota and Basidiomycota did show little or no change at the phylum level (Figure 3), although Basidiomycota significantly increased under water limitation when only considering the mineral soil (Figure S3). The categorization into functional guilds using FUNGuild (Nguyen *et al.* 2016) did also not provide any consistent patterns (data not shown). However, the significant shifts at lower taxonomic levels clearly demonstrated that a phylum-level survey largely ignores the massive physiological and ecological diversity within Ascomycota and Basidiomycota.

At class level, Agaricomycetes and Eurotiomycetes were more prevalent under water limitation (Figure 3), in particular when only considering the mineral horizon (Figure S3). Agaricomycetes contain saprobic, parasitic, and mutualistic species, but in forest ecosystems they mainly function as ectomycorrhizal symbionts of trees and primary wood-decayers (Hibbett *et al.* 2014). Investigations at lower levels revealed that typical saprobic Agaricomycetes did not change, whereas typical ectomycorrhizal Agaricomycetes responded either positively (e.g. *Inocybe*, *Rhizopogon*) or negatively (e.g. *Tricholoma*, *Craterellus*) to irrigation. The bi-directional response of known ectomycorrhizal species could be due to different capabilities to cope with water-limitation, or reflect the different stages of succession associated with compositional changes in the plant community as reported earlier (Herzog *et al.* 2014; see Supplementary Results for more details). It is also known that ectomycorrhizal fungi are vertically stratified and become more important in deeper soils where they mobilize nitrogen to be supplied to the plant roots (Lindahl *et al.* 2007; Rosling *et al.* 2003; Voříšková *et al.* 2014), likely explaining the stronger effect of irrigation on ectomycorrhizal groups such as the Agaricomycetes in the mineral soil (Figure S3). Generally, it can be hypothesized that ectomycorrhizal relationships are promoted under water limitation in order to protect the trees from desiccation and starvation (Brunner *et al.* 2015), although the impact of water limitation on ectomycorrhizal population is not entirely clear (van der Molen *et al.* 2011). We observed a general increase of Agaricomycetes under water limitation, but the results at lower levels show that such generalizations are difficult and factors such as vegetational shifts, stage of succession, and the individual capability to cope with water limitation are important.

Eurotiomycetes also tended to increase under water limitation (Figure 3, Figure S3). Members of the Eurotiomycetes occur as pathogens, symbionts, or saprobes and can degrade a wide variety of organic substrates (Geiser *et al.* 2015), which might give them advantages under water-limited conditions. At lower levels, we found several melanized dark-septate fungi of the Eurotiomycetes (Chaetothyriales) or Dothideomycetes (Capnodiales) that were increased under water limitation (see Supplementary Results). These fungi often occur in semi-arid and oligotrophic environments and are known to be tolerant towards desiccation and osmotic stress (Knapp *et al.* 2012). This observation further demonstrates that bacterial and fungal taxa able to establish in semi-arid ecosystems have

developed specific adaptation mechanisms to cope with desiccation, starvation, and other stresses associated with long-term water limitation.

Certain fungal groups such as the basidiomycetous classes Microbotryomycetes and Tremellomycetes as well as the ascomycetous classes Saccharomycetes and Leotiomyces increased under irrigation (Figure 3). Microbotryomycetes, Tremellomycetes, and Saccharomycetes are fungal classes comprising saprobic yeasts, fungal guilds that typically prefer moist environments rich in simple soluble nutrients (Choudhary & Johri 2009; Suh *et al.* 2006), but have also been shown to be the main fungal cellulose utilizers in forest soils (Štursová *et al.* 2012). These features could explain their increased abundance under increased primary production. Leotiomyces and its major polyphyletic subclade Helotiales are ecologically and functionally very diverse (Wang *et al.* 2006), but many members are saprobic and/or plant-associated, which might explain their increased abundance under a richer (in terms of biomass and vegetation cover) and compositionally different vegetation in the irrigated stands (Herzog *et al.* 2014; see Supplementary Results for more details).

Overall, it has to be noted that the response within the Ascomycota and Basidiomycota was fairly heterogeneous (Figure 2), suggesting that very specific, probably often vegetation-dependent shifts took place. Irrigation might have selected for taxa that are more competitive under altered vegetation, increased plant growth, higher nutrient availability, or enhanced occurrence of photosynthetic products within and outside of the roots in the rhizosphere (Philippot *et al.* 2013). The taxonomically heterogeneous response is not surprising, as fungal species are known to vary strongly in their carbon resource niches and host affinities (Baldrian 2009; Clemmensen *et al.* 2013), making it unlikely that higher-order taxonomic groups respond coherently. Not only substrate availability but also shifts in vegetation associated with irrigation have likely shifted the fungal species composition. Such shifts in vegetation upon irrigation with greater coverage of deciduous shrubs, increase of Scots pine but decrease of pubescent oak coverage have previously been demonstrated at this site (Herzog *et al.* 2014). Changes in vegetation can alter the quantity and quality of plant materials on one side, but also changes in the types of roots and root biomass on the other side, affecting the abundance and activities of certain microbial taxa (Koyama *et al.* 2014). Root-microbe interactions can either mitigate or

enhance effects of soil moisture on the soil microbiome (Pailler *et al.* 2014), highlighting the inherent importance of the host plant community in shaping soil microbial diversity.

In search of taxonomic groups with ecological coherence

There is increasing evidence that a certain degree of ecological coherence exists at higher phylogenetic levels and specific, often complex traits such as oxygenic photosynthesis or methane oxidation are phylogenetically fairly conserved (Martiny *et al.* 2013; Philippot *et al.* 2010). Other, usually less complex traits, such as the usage of simple carbon compounds, are often phylogenetically more dispersed (Martiny *et al.* 2013). Therefore, assessing phylum-level responses is of certain importance, but the diversity at all phylogenetic levels is relevant for ecosystem functioning. An exhaustive discussion of all detected effects is beyond the scope here, but we provide more details in the Supplementary Results and want to highlight three interesting scenarios where investigations at multiple taxonomic levels are crucial.

A first scenario is that subgroups within a phylum might show different response directions than the one observed at the phylum level, potentially leading to misinterpretations. This was the case in terms of Acidobacteria, a group of bacteria largely considered oligotrophic (Fierer *et al.* 2007; Kielak *et al.* 2016) and shown to increase under long-term water limitation in the present study (Figure 3) and by others (Curiel Yuste *et al.* 2014). However, different subgroups (Gp) showed a pronounced bivalent response to irrigation (Figure S4). Gp1 (Acidobacteriia), Gp2 (Solibacteres), and Gp4 (Chloracidobacteria) were more abundant in the dry plots, whereas Gp6 increased under irrigation. Based on these observations, we conclude that subgroups within Acidobacteria occupy different ecological niches. The presence of different response types has important implications for data interpretation. For example, Barnard *et al.* (2013) observed a strong decrease of Acidobacteria under short-term drought, which could be perceived as contradiction to the oligotroph-copiotroph hypothesis presented here; however, the vast majority of the acidobacterial sequences found by Barnard *et al.* were assigned to Gp6, which we and others (Gschwendtner *et al.* 2015; Naether *et al.* 2012; Navarrete *et al.* 2015) have found to behave rather copiotrophically.

A second scenario where multi-level investigations are important is one where the response of sensitive subgroups is masked by other abundant and more stable subgroups that dominate the effect at higher levels. This scenario emerged for the Bacteroidetes, which did not respond at the phylum level, because abundant classes including Saprospirae, Sphingobacteriia, and Flavobacteriia were unresponsive (Figure S4). However, the class Cytophagia down to the genus *Cytophaga*, an important group of cellulose utilizers (McBride *et al.* 2014), revealed a pronounced increase under irrigation.

In a third scenario, the higher-level group does not show any response since the response directions of different sensitive subgroups neutralize each other. This scenario can also occur in combination with scenario two. The highly diverse fungal phyla Ascomycota and Basidiomycota showed this phenomenon, hardly changing at the phylum level, but revealing significant contrasting responses already at class level (Figure 3). These scenarios can certainly propagate through the whole taxonomic hierarchy.

These examples highlight the need to carefully assess effects at different levels in order to get a more complete understanding of the system. In this context, it is important to keep in mind that given the substantial response heterogeneity within phylogenetically and metabolically diverse bacterial phyla such as Proteobacteria or Acidobacteria, generalizations regarding their copiotrophic and oligotrophic lifestyle and, thus, the trophic level of the environment under investigation (Smit *et al.* 2001), need to be treated with caution. Accordingly, this could also be one reason why there were no clear patterns when trying to summarize different fungal taxa into functional guilds.

Conclusion

This unique long-term irrigation study provides new insights into the extent that the soil microbiome could be modified in an ecosystem with a strong history of water limitation. Long-lasting increased irrigation was not only responsible for the shifts in the soil microbiome but also stimulated tree growth and compositional changes of the forest vegetation. Greater amounts of plant-derived inputs (e.g. litter fall and root biomass) associated with increased tree growth in the irrigated forest stands stimulated soil microbial activity coupled to pronounced shifts in the microbiome from largely oligotrophic to more copiotrophic lifestyles. Microbial groups benefitting from increased resource availabilities (litter,

rhizodeposits) thrived under irrigation, leading to enhanced soil organic matter mineralization and carbon respired from irrigated soils. The higher loss of respired C induced by these events was contrasted by higher primary production to the extent that it largely compensated for the increased SOM mineralization, resulting in similar soil C stocks. These findings have implications for our understanding of belowground C dynamics (e.g. long-term soil C stocks) under climate change, with the soil microbiome playing an integral role in these processes.

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Data accessibility

All quality-checked DNA sequences are available as Supplementary Data 1. The complete OTU table including bacteria and fungi is available as Supplementary Data 2. Other environmental data are available as Supplementary Data 3

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Figure legends

Figure 1. Irrigation effects on bacterial and fungal diversity along the soil depth profile. Changes in β -diversity were assessed by analysis of principal coordinates (PCO) based on Bray-Curtis similarities calculated from relative OTU abundances. PCO ordinations were generated separately for bacteria (B) and fungi (F), and overlaid using procrustes analysis (bacterial and fungal data points from the same sample are connected by lines; procrustes goodness of fit $m^2=0.09$, $P<0.001$; mantel test of underlying similarity matrices $r=0.81$, $P<0.001$). The 99% confidence ellipses for the centroids of each cluster are provided for bacteria and fungi separately. The variance explained by each PCO axis is given in parentheses (first for bacteria, second for fungi). Changes in α -diversity were assessed by calculating Shannon diversity indices based on iteratively rarefied OTU counts and visualized by boxplots (bacteria: upper panel; fungi: lower panel) including the individual values. Effect strengths were assessed by permutational analysis of variance (PERMANOVA) and are provided in Table 2. Org: organic F-horizon; Min 2: mineral soil (0 – 2 cm depth); Min 10: mineral soil (5 – 10 cm depth).

Figure 2. (a) Hierarchical (i.e. tree topology) taxonomic networks of the detected bacterial and fungal communities showing the OTU distribution across the different phyla. Nodes correspond to OTUs and node sizes correspond to their relative abundances (square root). Edges represent the taxonomic path from phylum to OTU level and OTUs were placed at the level of the lowest possible assignment. Floating nodes corresponds to OTUs that were unclassified at the phylum level. Individual networks are color-coded by their phylum-level assignment and are labeled with phylum name and relative abundance (phyla without abundance information accounted for less than 0.1%). (b) The same network topology as in panel (a), but nodes sizes correspond to the positive relative change (z-transformed) in abundance in either the dry (left) or irrigated (right) stands. Node colors correspond to the level of significance going from highly significant (red/blue) to not significant (grey). A soft threshold using a color gradient rather than a hard cutoff was used for denoting the level of

significance (nodes with $q \geq 0.15$ are completely grey and nodes with $q \leq 0.05$ are completely red/blue).

Figure 3. Relative change (z-transformed) in abundance of all bacterial and fungal phyla (upper panel) as well as the major proteobacterial, ascomycetous and basidiomycetous classes (lower panel). The first vertical panel represents the relative change in abundance from the overall mean, including the average change (vertical lines) and the corresponding standard error (boxes, $n=4$). The second vertical panel shows the strength of the irrigation effect as assessed by permutational analysis of variance (PERMANOVA) including the F-ratio (visualized by differently sized circles) as well as the uncorrected (p) and corrected (q) levels of significance. The third vertical panel shows the degree of depth-dependency of the irrigation effect (irrigation $\times$ depth interaction) as assessed by PERMANOVA including the F-ratio (visualized by differently sized circles) as well as the uncorrected (p) and corrected (q) levels of significance. The fourth vertical panel shows the relative abundance (percent reads standardized by domain) as well as the number of OTUs for each investigated taxon, both metrics being visualized by differently sized circles.

Supplementary Figure 1. (a) Monthly mean volumetric water content (%) of the irrigated (dashed blue) and the control (red) plots over the experimental period (2003 – 2013). Annual precipitation (mm) and the applied annual irrigation (b). Irrigation periods are indicated as grey bars. The figure was adapted from Herzog *et al.* (2014).

Supplementary Figure 2. Rarefaction (solid lines) and extrapolation (dashed lines) curves of the observed bacterial and fungal OTU richness at different soil depths in the irrigated and dry plots. Samples from the same treatment and soil horizon were pooled prior to analysis. The solid circles represent the actually observed value, whereas the shaded regions represent the 95% confidence intervals obtained by bootstrapping with 200 replications. Org: organic F-horizon; Min 2: mineral soil (0 – 2 cm depth); Min 10: mineral soil (5 – 10 cm depth).

Supplementary Figure 3. Relative (standardized and scaled) change in abundance of all bacterial and fungal phyla (upper panel) as well as the major proteobacterial, ascomycetous and basidiomycetous classes (lower panel). These results are equivalent to Figure 2, but only based on the mineral soil (excluding the organic horizon). See legend of Figure 2 for further details.

Supplementary Figure 4. Relative (standardized and scaled) change in abundance of all bacterial and fungal taxa from phylum to genus level. These results are equivalent to Figure 2 and provide the complete corresponding statistics for all detected phyla, classes, orders, families and genera. The taxa are ordered hierarchically with lower taxonomic levels showing increasingly stronger indentation. Taxon labels (green for bacteria and red for fungi) of different phyla are separated by horizontal lines. See legend of Figure 2 for further details.

Supplementary Figure 5. Relative (standardized and scaled) change in abundance of all bacterial and fungal taxa from phylum to genus level. These results are equivalent to Supplementary Figure 4, but only based on the mineral soil (excluding the organic horizon). See legend of Supplementary Figure 4 for further details.

Table 1. Abiotic and biotic site characteristics of dry and irrigated plots of the Pfywald experimental installation.

	Dry plots (mean ± SE)			Irrigated plots (mean ± SE)			ANOVA ¹		
	ORG	MIN 0-2 cm	MIN 5-10 cm	ORG	MIN 0-2 cm	MIN 5-10 cm	Irrigation	Depth	Interaction
Stratified measures by soil horizon									
Soil chemistry									
pH	NA	5.3±0.2	6.9±0.0	NA	6.5±0.0	6.9±0.1	***	***	***
C [%]	36.9±1.2	15.8±1.4	4.3±0.3	19.6±2.1	13.4±1.6	5.8±0.7	***	***	***
N [%]	1.2±0.1	0.6±0.1	0.2±0.0	0.6±0.0	0.6±0.1	0.3±0.0	**	***	***
C/N ratio	32±3	25±0	22±0	30±3	24±1	23±2	ns	***	ns
Microbial biomass									
Total biomass [µg DNA g ⁻¹ soil dw]	33±7	21±5	19±5	25±5	29±4	20±4	ns	ns	ns
Bacterial biomass [nmol PLFA g ⁻¹ soil dw]	638±127	318±18	98±5	646±38	269±16	114±9	ns	***	ns
Fungal biomass [nmol PLFA g ⁻¹ soil dw]	99±20	41±3	12±1	102±2	36±1	13±1	ns	***	ns
Fungal to bacterial PLFA biomass ratio	0.16±0.01	0.13±0.00	0.12±0.01	0.16±0.01	0.13±0.00	0.12±0.00	ns	***	ns
Microbial activity									
Basal respiration [mg CO ₂ -C g C ⁻¹ d ⁻¹]	1.6±0.3	1.5±0.2	7.8±0.3	4.3±0.6	4.9±1.0	11.6±1.0	***	***	ns
C-mineralization [mg CO ₂ -C g C ⁻¹ month ⁻¹]	28.8±3.7	28.9±5.3	68.3±2.0	54.8±5.1	55.7±9.1	77.4±6.5	***	***	ns
Bulk measures per plot									
Soil properties									
C-stock litter and below-ground C at 0-10 cm [kg C m ⁻²]			5.33±0.57			6.35±0.90	ns	-	-
Soil respiration [µmol CO ₂ -C m ⁻² s ⁻¹]			3.29±0.24			5.82±0.34	***	-	-
Volumetric water content (%)			27.8±0.7			34.3±0.6	*	-	-
Fine root properties									
Fine root biomass at 0-10 cm [kg m ⁻²] ²			0.266±0.038			0.392±0.047	*	-	-
Fine root δ ¹³ C cellulose [‰] ²			-23.9±0.1			-24.8±0.2	**	-	-
Vegetation									
Litter fall [kg m ⁻² yr ⁻¹]			0.313±0.031			0.462±0.041	*	-	-
Tree biomass [kg m ⁻²]			6.85±0.25			7.91±0.61	ns	-	-
Yearly tree biomass increment [kg m ⁻² yr ⁻¹]			0.06±0.00			0.13±0.01	**	-	-
Crown cover [%]			57±3			71±1	**	-	-
Plant richness [species per plot] ²			38±1			39±2	ns	-	-

¹ Effects of irrigation, soil depth and their interaction were assessed by analysis of variance (ANOVA; ns, not significant, * P<0.05, **P<0.01, ***P<0.001).

² Data based on Herzog et al. 2014.

Table 2. Irrigation effects on bacterial and fungal α - and β -diversity along the soil depth profile.

	Bacteria		Fungi	
	α -diversity ¹	β -diversity ¹	α -diversity	β -diversity
All horizons				
Irrigation	3.65 (0.072)	3.40 (<0.001)	0.29 (0.600)	3.85 (<0.001)
Soil horizon	0.89 (0.429)	2.88 (<0.001)	0.07 (0.929)	2.83 (<0.001)
Irrigation $\times$ soil horizon	0.31 (0.738)	1.06 (0.305)	1.92 (0.164)	1.10 (0.265)
Mineral horizons only				
Irrigation	4.28 (0.060)	2.83 (<0.001)	4.81 (0.051)	4.11 (<0.001)
Soil horizon	1.86 (0.201)	1.77 (0.010)	0.00 (0.972)	1.49 (0.081)
Irrigation $\times$ soil horizon	0.00 (0.962)	1.04 (0.325)	3.27 (0.095)	0.85 (0.666)

¹ Changes in microbial diversity were assessed by univariate (α -diversity measured as Euclidean distances of Shannon diversity indices) and multivariate (β -diversity measured as Bray-Curtis similarities) permutational ANOVA (PERMANOVA). Shannon diversity indices were calculated from evenly rarefied OTU abundance matrices in order to avoid biases from different sampling efforts. Values in the table represent the pseudo-F ratio and the level of significance (P) in brackets. Values at P<0.05 are shown in bold. Only the depth-dependent effects on β -diversity were influenced by differences in dispersion with the organic horizon showing higher dispersion (assessed by PERMDISP, data not shown).

Figure 1

99x56mm (300 x 300 DPI)

Figure 2

210x248mm (300 x 300 DPI)

Figure 3

210x247mm (300 x 300 DPI)