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

2

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20 **Abstract**

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

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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
59 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.

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

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

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91 introduce significant shifts to the forest soil microbiome that are in agreement with the current
92 understanding of oligotrophic and copiotrophic life strategies (Fierer *et al.* 2007). We hypothesized
93 that increased litter fall and stimulated root growth under irrigation promote the occurrence of
94 copiotrophs such as Proteobacteria and Bacteroidetes (Fierer *et al.* 2007), casual mesotrophic root
95 invaders such as zygomycete fungi (Dix & Webster 2012; Richardson 2009), and early litter saprobes
96 such as Ascomycota (Voriskova & Baldrian 2013), whereas water-limited conditions favor
97 oligotrophic, metabolically versatile, or drought-tolerant bacteria such as Acidobacteria,
98 Actinobacteria, Gemmatimonadetes (DeBruyn *et al.* 2011; Fierer *et al.* 2007; Hanada & Sekiguchi
99 2014; Kielak *et al.* 2016; Mohammadipanah & Wink 2015; Rosenberg *et al.* 2014) as well as
100 ectomycorrhizal fungi such as Basidiomycota as a consequence of a shift in vegetation (Herzog *et al.*
101 2014) and increased symbiosis to protect the plants from desiccation and starvation (Brunner *et al.*
102 2015). Changes in quantity and/or quality of C inputs and shifts in bacterial and fungal community
103 structures following long-term irrigation of dry soils might in turn alter soil ecosystem functioning
104 (SOM mineralization and transformation) with important implications for the fate of long-term soil C
105 stocks under climate change.
106
107 **Material and methods**
108 *Experimental set up and soil sampling*
109 The Pfyn forest long-term irrigation experiment is situated in the Rhone Valley of Switzerland
110 (46°18'N, 7°37'E, 615 m.a.s.l.), in a Scots pine (*Pinus sylvestris*) forest with occasional interspersed
111 pubescent oak (*Quercus pubescens*). The study area, including 899 numbered and geo-referenced pine
112 trees, was divided into eight plots of 25 x 40 m (1000 m^2) with 5 m buffer areas between and around
113 each plot (Dobbertin *et al.* 2010; Herzog *et al.* 2014). The plots were aligned side by side along a
114 channel fed by the Rhone River, from where water was taken to irrigate four randomly selected plots
115 (further termed "irrigated"). Four plots were left untreated (further termed "dry"). Irrigation was
116 started for the first time in spring 2003. The irrigation system was activated on rainless nights during
117 the vegetation period (May-October), doubling the annual rainfall amount (Feichtinger *et al.* 2014).

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118 Soils were sampled with a quantitative soil pit approach using a metal frame of 20 x 20 cm in
119 October 2012 at the end of the irrigation period. Four replicate samples were collected at four different
120 locations in each of the eight plots (at least 5 m distance from the plot edge) at three soil depths, i.e.
121 the organic F-Horizon (Org) as well as at 0-2 cm depth (Min2) and 5-10 cm depth (Min10) of the
122 mineral soil. The four individual replicates per plot and depth were pooled for each study plot
123 separately, leaving the independent plots as the level of replication. This gave a total of 24 soil
124 samples (2 treatments × 4 independent plots × 3 soil horizons). The fresh soil samples were
125 homogenized using a 2 mm sieve. For estimation of soil microbial biomass and carbon mineralization,
126 soils were processed directly. For PLFA and DNA analysis, soils were immediately frozen and kept at
127 - 80°C. For soil analysis, subsamples were dried at 60°C and ground with a ball mill.
128
129 *Basic soil and plant characteristics*
130 Volumetric soil water content was monitored hourly using time domain reflectometry (Tektronix
131 1502B cable tester, Beaverton, OR) at 10 cm soil depth at four different locations in irrigated and dry
132 plots. The mean volumetric water content in the soil significantly increased from 27.8% in the dry
133 plots to 34.3% in the irrigated plot (Herzog *et al.* 2014; Supplementary Figure S1). Soil pH was
134 determined in 0.01 M $CaCl_2$. Total carbon and nitrogen content were measured using an automated
135 elemental analyser (Euro EA 3000, HEKAtech, Germany). Soil organic C (SOC) stock was
136 determined by multiplying SOC concentrations with the bulk densities and the volume of the
137 corresponding soil layers considering the stone contents.
138 Fine root sampling was executed twice, i.e. in May 2003 and in May 2012 each time before the
139 irrigation started. The fine roots were sampled by sampling four soil cores at a distance of 1 m from
140 each of three trees per plot with soil coring cylinder (Ø 45 mm) and analyzed according to Herzog *et*
141 *al.* (2014). Cellulose extraction from fine roots and ^{13}C measurements of the extracted cellulose was
142 performed following the protocol of Herzog *et al.* (2014).
143 Litter fall was collected by five litter-traps of 50 x 50 cm placed on the ground in each of the eight
144 plots (total n=40). The litter material was sampled in autumn and spring, and dried at 60°C for two
145 days before weighing. The above-ground tree biomass was estimated by using allometric relationships

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146 (stem diameter, tree height; Etzold *et al.* 2014) and annual increments of the above-ground volume of
147 all individual trees were compared with the tree volume of 2002. Crown cover was recorded in 2008
148 according to Dobbertin *et al.* (2010). Plant richness was recorded in May 2012 and estimated
149 according to Herzog *et al.* (2014).
150

151 *Microbial biomass carbon, carbon mineralization and respiration measurements*

152 Phospholipid fatty acids (PLFA) were extracted and measured using gas chromatography–mass
153 spectrometry (GC-MS) as described previously (Frey *et al.* 2006; Hagedorn *et al.* 2013). The fatty
154 acids 18:2ω6,9 and 18:1ω9 were used as a biomarker for fungi (Bååth & Anderson 2003), while the
155 fatty acids 16:1ω7, cy17:0, 18:1ω7, cy19:0, i15:0, a15:0, i16:0, i17:0, a17:0 were markers for bacteria
156 (Zogg *et al.* 1997).

157 Soil respiration was measured in the field using an infrared gas analyser (IRGA) on a monthly basis
158 in 2012 according to Guelland *et al.* (2013). Soil respiration is directly measured in the field and
159 serves as the total CO_2 emissions from soils including heterotrophic and autotrophic respiration. In this
160 system, it can be expected that a significant amount of the respired CO2 is actually coming from the
161 roots of the trees and not from the soil microbiota. Basal respiration was determined under field moist
162 conditions (i.e. different for dry and irrigated soils) in a closed loop system with a CO_2 analyzer (LI-
163 COR-840) at 20°C. Potential C mineralization was measured under standardized moisture conditions
164 (adjusted to 50% water holding capacity) on a weekly basis using the same method as described
165 above.
166

167 *Pyrosequencing of bacterial and fungal ribosomal markers*

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

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174 using the GS-FLX Titanium technology (Roche 454 Life Sciences, Brandford, CT, USA) at the
175 Genome Quebec Innovation Center, Montreal, Canada.
176 Quality control, clustering into operational taxonomic units (OTUs), and taxonomic classification
177 of bacterial and fungal reads was performed according to Hartmann *et al.* (2014). The 454
178 pyrosequencing reads were deposited in the European Nucleotide Archive under the accession number
179 PRJEB14824. A FASTA-formatted file containing all high quality sequences analyzed in this study is
180 provided as Supplementary Data 1.
181
182 *Statistical analyses of microbial data*
183 Alpha-diversity estimates included the Shannon diversity index based on iteratively rarified (at
184 3270 bacterial and 2312 fungal reads) OTU abundance data calculated in MOTHUR (Schloss *et al.*
185 2009) as well as rarefaction interpolation and extrapolation analysis of the observed richness (Chao *et*
186 *al.* 2014) using the package iNEXT (Hsieh *et al.* 2013) in R (R Core Team 2014). Beta-diversity was
187 measured by Bray-Curtis similarities calculated from standardized square-root transformed OTU
188 abundances. Square-root transformation of standardized data, also known as Hellinger transformation,
189 is used to downweight the contributions of quantitatively dominant taxa to the similarities calculated
190 between samples without losing the influence of less abundant taxa (Clarke & Warwick 2001).
191 Differences in β-diversity across samples were examined by principal coordinate analysis (PCO) using
192 the *cmdscale* function in R. Bacterial and fungal PCO ordinations and underlying Bray-Curtis
193 resemblance matrices were compared using Procrustes analysis and Mantel testing, respectively, as
194 implemented in the R package vegan (Oksanen *et al.* 2016). Irrigation effects along the soil depth
195 profile on α- and β-diversity were quantified using univariate and multivariate permutational analysis
196 of variance (PERMANOVA, Anderson 2001), respectively. Homogeneity of variances across
197 treatments and soil horizons were examined using permutational analysis of multivariate dispersions
198 (PERMDISP, Anderson 2006). PERMANOVA and PERMDISP were performed using the
199 homonymous routines implemented in PRIMER6+ (Clarke & Gorley 2006). Taxon-level responses to
200 irrigation were assessed at different taxonomic levels from phylum to OTU using univariate
201 PERMANOVA as implemented by the *adonis* function in the R package vegan. In order to retrieve

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202 taxa showing a universal response to irrigation or long-term water limitation, relative abundance data
203 were centered by depth and scaled (combined also known as z-transformation) prior to
204 PERMANOVA and visualization. Adjustments for multiple hypothesis testing for this type of data is
205 potentially problematic and lead to an inflation of false negatives, since the basic assumption of
206 uniform p-value distribution under the null hypothesis is violated for sparse count data, resulting in a
207 bimodal p-value distribution (Storey & Tibshirani 2003). We performed multiple testing adjustment
208 using the false discovery rate correction according to Storey & Tibshirani (2003) implemented in the
209 R package qvalue (Storey *et al.* 2015), but provide the exact p-value and the adjusted q-value
210 alongside for all tests. Hierarchical taxonomic networks were generated based on the taxonomic path
211 of each OTU and the OpenCL-accelerated Allegro Fruchterman–Reingold algorithm in CYTOSCAPE
212 3.3 (Shannon *et al.* 2003) as described previously (Frey *et al.* 2016). Information on treatment-
213 sensitive OTUs were subsequently mapped onto these networks.

214

215 **Results and Discussion**

216 *Primary production and associated changes in microbial diversity*

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

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

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

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

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

273

274 *Long-term irrigation induced shifts from oligotrophic to copiotrophic lifestyles*

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

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

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

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

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

326 Planctomycetes also tended to be more abundant under irrigation (Figure 3), but the literature
327 provides equivocal results regarding their response to precipitation change (Bachar *et al.* 2010;
328 Barnard *et al.* 2013; Bouskill *et al.* 2013; von Rein *et al.* 2016; Waring & Hawkes 2015; Zeng *et al.*
329 2016), likely depending on what subgroups are detected. Planctomycetes are usually slow growing,
330 aerobic bacteria that have several unusual cell characteristics (Fuerst & Sagulenko 2011). The lack of
331 cultured representatives still limits our understanding of these bacteria, making it difficult to speculate
332 about potential adaptation mechanisms under water limitation and long-term irrigation; however, it has
333 been suggested some members can grow on complex heteropolysaccharides such as from decaying
334 wood (Wang *et al.* 2015), which could be a reason for their prevalence under increased primary
335 production in the irrigated plots (Supplementary Results).

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

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

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342 factors such as metabolic versatility to degrade complex organic compounds, higher C-use efficiency,
343 and tolerance towards desiccation and nutrient limitations through cellular modifications (osmotic
344 protectants, dormancy) are important traits for survival and growth (Schimel *et al.* 2007).
345 Actinobacteria, for example, are ubiquitous and frequently saprophytic organisms able to degrade
346 recalcitrant carbon, play a vital role in the soil C-cycle, and have been shown to be highly resistant
347 towards desiccation and C starvation (Bull 2011; Mohammadipanah & Wink 2015; Rosenberg *et al.*
348 2014; Ventura *et al.* 2007). The ability to decompose recalcitrant compounds is an important trait in
349 arid systems when more readily available substrates are rather limited. Furthermore, many members of
350 the Actinobacteria are capable of spore formation and filamentous growth, which facilitates survival
351 under conditions of low hydraulic connectivity in unsaturated soils (Wolf *et al.* 2014). With these
352 capabilities, Actinobacteria are known to compete well under arid conditions, correlate negatively with
353 soil moisture (Zeng *et al.* 2016), and appear to be adapted to semi-arid soil environments (Banerjee *et*
354 *al.* 2016; Barnard *et al.* 2013; Bouskill *et al.* 2013; Chodak *et al.* 2015; Curiel Yuste *et al.* 2014;
355 Felsmann *et al.* 2015; von Rein *et al.* 2016; Waring & Hawkes 2015), supporting our observations
356 (Figure 3).

357 Gemmatimonadetes are abundant but physiologically poorly studied soil bacteria, and
358 biogeographic surveys suggest a strong adaption to environments of low moisture contents (DeBruyn
359 *et al.* 2011). The presence of Gemmatimonadetes in arid and often extremely nutrient-limited
360 environments such as cave walls (Pašić *et al.* 2010; Zhou *et al.* 2007), weathering rocks (Cockell *et al.*
361 2009), or subglacial sediments (Rime *et al.* 2015; Rime *et al.* 2016) indicates a strong tolerance
362 towards desiccation and adaptation to oligotrophic conditions (Hanada & Sekiguchi 2014), which is
363 again in agreement with our hypothesized framework. However, the impact of water availability on
364 the abundance and distribution of Gemmatimonadetes is not yet understood as previous studies have
365 found both positive (Curiel Yuste *et al.* 2014; DeBruyn *et al.* 2011) and negative (Chodak *et al.* 2015;
366 Zeng *et al.* 2016) relationships with water limitation.

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

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370 availability (Fierer *et al.* 2007; Koyama *et al.* 2014) and to increase during long-term water limitation
371 in a Mediterranean forest (Curiel Yuste *et al.* 2014), supporting our observations (Figure 3).
372 Armatimonadetes is a more recently discovered, moderately abundant but phylogenetically diverse
373 bacterial phylum with very few cultured representatives (Lee *et al.* 2014). Few common phenotypic
374 characteristics have so far emerged, but all cultivated members share an aerobic oligotrophic
375 metabolism that, again, is in agreement with the copiotroph-oligotroph framework under water
376 limitation and irrigation. In agreement with our observations (Figure 3), Armatimonadetes were
377 negatively correlated with soil moisture and mean annual precipitation along a latitudinal gradient in
378 China (Zeng *et al.* 2016). Taken together, Actinobacteria, Gemmatimonadetes, Acidobacteria, and
379 Armatimonadetes appear to be well adapted to semi-arid soil ecosystems and potentially get
380 outcompeted under increased primary production after long-term irrigation.
381 Other bacterial phyla commonly found in soil such as Chloroflexi (4.0%), Firmicutes (0.3%) and
382 Verrucomicrobia (0.6%) showed no consistent responses to irrigation (Figure 3). Previous studies also
383 reported rather equivocal results of these groups in response to water limitation and precipitation. For
384 example, some studies suggested that Verrucomicrobia follow a more copiotrophic lifestyle with
385 preference for moist soils (Barnard *et al.* 2013; Chodak *et al.* 2015; Curiel Yuste *et al.* 2014), whereas
386 others have suggested a more oligotrophic life-strategy of Verrucomicrobia in respect to water
387 availability (Bachar *et al.* 2010; von Rein *et al.* 2016). Similar reports have been found for Firmicutes,
388 documenting negative (Bouskill *et al.* 2013), positive (Bachar *et al.* 2010; Li *et al.* 2016) or no
389 association (Barnard *et al.* 2013; Curiel Yuste *et al.* 2014) with arid conditions, such that it remains
390 challenging to classify them according to the copiotroph-oligotroph hypothesis (Fierer *et al.* 2007). A
391 more resistant life strategy in relation to water limitation and irrigation was also reported for the
392 phylum Chloroflexi (Bachar *et al.* 2010; Barnard *et al.* 2013; Zeng *et al.* 2016). However, these
393 equivocal results also suggest that surveying phylum-level abundances is a simplistic view that ignores
394 potentially heterogeneous responses at lower taxonomic levels (see discussion below).
395 Bacteroidetes is another bacterial phylum commonly considered to feature copiotrophic lifestyles
396 (Fierer *et al.* 2007), usually well represented in well-watered soils, and reported to decrease in
397 numbers under drought (Chodak *et al.* 2015; Curiel Yuste *et al.* 2014). However, this phylum did not

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398 respond to irrigation when all soil horizons were included in the analysis (Figure 3). Since inter-
399 sample dispersion was highest in the organic horizon and decreased with depth (data not shown), some
400 underlying shifts that were confined to the mineral horizon were masked and became evident when
401 excluding the organic layer from the analysis (Figure S3). Whereas all above observations also hold
402 true when only considering the mineral soil, the phylum Bacteroidetes revealed a strong increase under
403 irrigation in the mineral soil, further confirming the copiotroph-oligotroph hypothesis. It was also
404 notable that effects at class level, for example for Beta-, Gamma-, and Deltaproteobacteria became
405 more pronounced when considering the mineral soil only (Figure S3). At this point, it is also
406 noteworthy to mention that certain microbial populations could be brought into the soil through the
407 irrigation system that was fed with water from the Rhone River. However, it is unlikely that microbial
408 populations adapted to aquatic systems and inoculated at a relatively low abundance will establish and
409 thrive in forest soils such that they override the indigenous community to a degree that such strong
410 treatment effects are observed.
411
412 *Response of fungal decomposers and symbionts to irrigation*
413 We hypothesized that increased litter fall under irrigation promotes the occurrence of saprobic
414 fungi (predominantly Ascomycota), whereas water limitation favors ectomycorrhizal relationships
415 (predominantly Basidiomycota). Among the decomposers, it has been suggested that rhizomorph and
416 cord-forming Basidiomycota rapidly colonize fresh litter under moist conditions, but that drought-
417 tolerant dark-septate Ascomycota predominate over Basidiomycota as both plant symbionts and
418 decomposers in arid environments (Lee Taylor & Sinsabaugh 2015). In the present study, Ascomycota
419 and Basidiomycota did show little or no change at the phylum level (Figure 3), although
420 Basidiomycota significantly increased under water limitation when only considering the mineral soil
421 (Figure S3). The categorization into functional guilds using FUNGuild (Nguyen *et al.* 2016) did also
422 not provide any consistent patterns (data not shown). However, the significant shifts at lower
423 taxonomic levels clearly demonstrated that a phylum-level survey largely ignores the massive
424 physiological and ecological diversity within Ascomycota and Basidiomycota.

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

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

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

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

466 Overall, it has to be noted that the response within the Ascomycota and Basidiomycota was fairly
467 heterogeneous (Figure 2), suggesting that very specific, probably often vegetation-dependent shifts
468 took place. Irrigation might have selected for taxa that are more competitive under altered vegetation,
469 increased plant growth, higher nutrient availability, or enhanced occurrence of photosynthetic products
470 within and outside of the roots in the rhizosphere (Philippot *et al.* 2013). The taxonomically
471 heterogeneous response is not surprising, as fungal species are known to vary strongly in their carbon
472 resource niches and host affinities (Baldrian 2009; Clemmensen *et al.* 2013), making it unlikely that
473 higher-order taxonomic groups respond coherently. Not only substrate availability but also shifts in
474 vegetation associated with irrigation have likely shifted the fungal species composition. Such shifts in
475 vegetation upon irrigation with greater coverage of deciduous shrubs, increase of Scots pine but
476 decrease of pubescent oak coverage have previously been demonstrated at this site (Herzog *et al.*
477 2014). Changes in vegetation can alter the quantity and quality of plant materials on one side, but also
478 changes in the types of roots and root biomass on the other side, affecting the abundance and activities
479 of certain microbial taxa (Koyama *et al.* 2014). Root-microbe interactions can either mitigate or

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480 enhance effects of soil moisture on the soil microbiome (Pailler *et al.* 2014), highlighting the inherent
481 importance of the host plant community in shaping soil microbial diversity.
482

483 *In search of taxonomic groups with ecological coherence*

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

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

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

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

519 These examples highlight the need to carefully assess effects at different levels in order to get a
520 more complete understanding of the system. In this context, it is important to keep in mind that given
521 the substantial response heterogeneity within phylogenetically and metabolically diverse bacterial
522 phyla such as Proteobacteria or Acidobacteria, generalizations regarding their copiotrophic and
523 oligotrophic lifestyle and, thus, the trophic level of the environment under investigation (Smit *et al.*
524 2001), need to be treated with caution. Accordingly, this could also be one reason why there were no
525 clear patterns when trying to summarize different fungal taxa into functional guilds.

526

527 *Conclusion*

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

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535 rhizodeposits) thrived under irrigation, leading to enhanced soil organic matter mineralization and
536 carbon respired from irrigated soils. The higher loss of respired C induced by these events was
537 contrasted by higher primary production to the extent that it largely compensated for the increased
538 SOM mineralization, resulting in similar soil C stocks. These findings have implications for our
539 understanding of belowground C dynamics (e.g. long-term soil C stocks) under climate change, with
540 the soil microbiome playing an integral role in these processes.
541
542
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551
552 **Data accessibility**

553 All quality-checked DNA sequences are available as Supplementary Data 1. The complete OTU table
554 including bacteria and fungi is available as Supplementary Data 2. Other environmental data are
555 available as Supplementary Data 3
556
557

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558 **References**

559 Anderegg WR, Kane JM, Anderegg LD (2013) Consequences of widespread tree mortality triggered by drought
560 and temperature stress. *Nature Climate Change* **3**, 30-36.

561 Bååth E, Anderson TH (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological
562 and PLFA-based techniques. *Soil Biology & Biochemistry* **35**, 955-963.

563 Bachar A, Al-Ashhab A, Soares MIM*, et al.* (2010) Soil microbial abundance and diversity along a low
564 precipitation gradient. *Microbial Ecology* **60**, 453-461.

565 Baldrian P (2009) Ectomycorrhizal fungi and their enzymes in soils: is there enough evidence for their role as
566 facultative soil saprotrophs? *Oecologia* **161**, 657-660.

567 Banerjee S, Helgason B, Wang L*, et al.* (2016) Legacy effects of soil moisture on microbial community structure
568 and N2O emissions. *Soil Biology and Biochemistry* **95**, 40-50.

569 Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme
570 desiccation and rewetting. *The ISME Journal* **7**, 2229-2241.

571 Bouskill NJ, Lim HC, Borglin S*, et al.* (2013) Pre-exposure to drought increases the resistance of tropical forest
572 soil bacterial communities to extended drought. *The ISME Journal* **7**, 384-394.

573 Brown CT, Hug LA, Thomas BC*, et al.* (2015) Unusual biology across a group comprising more than 15% of
574 domain Bacteria. *Nature* **523**, 208-211.

575 Brunner I, Herzog C, Dawes M, Arend M, Sperisen C (2015) How tree roots respond to drought. *Frontiers in*
576 *Plant Science* **6**.

577 Brzostek ER, Blair JM, Dukes JS*, et al.* (2012) The effect of experimental warming and precipitation change on
578 proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal. *Global Change Biology*
579 **18**, 2617-2625.

580 Bull AT (2011) Actinobacteria of the extremobiosphere. In: *Extremophiles Handbook* (ed. Horikoshi K), pp.
581 1203-1240. Springer Japan, Tokyo.

582 Chao A, Gotelli NJ, Hsieh T*, et al.* (2014) Rarefaction and extrapolation with Hill numbers: a framework for
583 sampling and estimation in species diversity studies. *Ecological Monographs* **84**, 45-67.

584 Chodak M, Gołębiewski M, Morawska-Płoskonka J, Kuduk K, Niklińska M (2015) Soil chemical properties
585 affect the reaction of forest soil bacteria to drought and rewetting stress. *Annals of Microbiology* **65**, 1627-1637.

586 Choudhary DK, Johri BN (2009) Basidiomycetous yeasts: current status. In: *Yeast Biotechnology: Diversity and*
587 *Applications* (eds. Satyanarayana T, Kunze G), pp. 19-46. Springer Netherlands, Dordrecht.

588 Clarke KR, Gorley RN (2006) *PRIMER v6: User Manual/Tutorial*, 6 edn. PRIMER-E, Plymouth.

589 Clarke KR, Warwick RM (2001) *Changes in marine communities: an approach to statistical analysis and*
590 *interpretation (3rd edition)* PRIMER-E Ltd., Plymouth, UK.

591 Clemmensen KE, Bahr A, Ovaskainen O*, et al.* (2013) Roots and associated fungi drive long-term carbon
592 sequestration in boreal forest. *Science* **339**, 1615-1618.

593 Cockell CS, Olsson K, Knowles F*, et al.* (2009) Bacteria in weathered Basaltic glass, Iceland. *Geomicrobiology*
594 *Journal* **26**, 491-507.

595 Cregger MA, Schadt CW, McDowell NG, Pockman WT, Classen AT (2012) Response of the soil microbial
596 community to changes in precipitation in a semiarid ecosystem. *Applied and Environmental Microbiology* **78**,
597 8587-8594.

For Review Only

598 Curiel Yuste J, Fernandez-Gonzalez AJ, Fernandez-Lopez M, *et al.* (2014) Strong functional stability of soil
599 microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline
600 precipitation. *Soil Biology and Biochemistry* **69**, 223-233.

601 de Vries FT, Liiri ME, Bjornlund L, *et al.* (2012) Land use alters the resistance and resilience of soil food webs
602 to drought. *Nature Climate Change* **2**, 276-280.

603 DeBruyn JM, Nixon LT, Fawaz MN, Johnson AM, Radosevich M (2011) Global biogeography and quantitative
604 seasonal dynamics of Gemmatimonadetes in soil. *Applied and Environmental Microbiology* **77**, 6295-6300.

605 Dix NJ, Webster J (2012) *Fungal Ecology* Springer Science & Business Media, London, UK.

606 Dobbertin M, Eilmann B, Bleuler P, *et al.* (2010) Effect of irrigation on needle morphology, shoot and stem
607 growth in a drought-exposed Pinus sylvestris forest. *Tree Physiology* **30**, 346-360.

608 Etzold S, Waldner P, Thimonier A, Schmitt M, Dobbertin M (2014) Tree growth in Swiss forests between 1995
609 and 2010 in relation to climate and stand conditions: recent disturbances matter. *Forest Ecology and*
610 *Management* **311**, 41-55.

611 Evans SE, Wallenstein MD (2014) Climate change alters ecological strategies of soil bacteria. *Ecology Letters*
612 **17**, 155-164.

613 Evans SE, Wallenstein MD, Burke IC (2014) Is bacterial moisture niche a good predictor of shifts in community
614 composition under long-term drought? *Ecology* **95**, 110-122.

615 Feichtinger LM, Eilmann B, Buchmann N, Rigling A (2014) Growth adjustments of conifers to drought and to
616 century-long irrigation. *Forest Ecology and Management* **334**, 96-105.

617 Felsmann K, Baudis M, Gimbel K, *et al.* (2015) Soil bacterial community structure responses to precipitation
618 reduction and forest management in forest ecosystems across Germany. *PLoS ONE* **10**, e0122539.

619 Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* **88**,
620 1354-1364.

621 Fierer N, Lauber CL, Ramirez KS, *et al.* (2012) Comparative metagenomic, phylogenetic and physiological
622 analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* **6**, 1007-1017.

623 Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial
624 competition? *Soil Biology and Biochemistry* **35**, 837-843.

625 Frey B, Pesaro M, Rüdt A, Widmer F (2008) Dynamics of bacterial communities in bulk and poplar rhizosphere
626 soil contaminated with heavy-metals. *Environ. Microbiol* **10**, 1433-1449.

627 Frey B, Rime T, Phillips M, *et al.* (2016) Microbial diversity in European alpine permafrost and active layers.
628 *FEMS Microbiology Ecology* **92**, 1-17.

629 Frey B, Stemmer M, Widmer F, Luster J, Sperisen C (2006) Microbial activity and community structure of a soil
630 after heavy metal contamination in a model forest ecosystem. *Soil Biology & Biochemistry* **38**, 1745-1756.

631 Fuerst JA, Sagulenko E (2011) Beyond the bacterium: planctomycetes challenge our concepts of microbial
632 structure and function. *Nature Reviews Microbiology* **9**, 403-413.

633 Geiser DM, LoBuglio KF, Gueidan C (2015) Pezizomycotina: Eurotiomycetes. In: *The Mycota VII: Systematics*
634 *and Evolution Part B* (eds. McLaughlin JD, Spatafora WJ), pp. 121-141. Springer Berlin Heidelberg, Berlin,
635 Heidelberg.

636 Goldfarb KC, Karaoz U, Hanson CA, *et al.* (2011) Differential growth responses of soil bacterial taxa to carbon
637 substrates of varying chemical recalcitrancechod. *Frontiers in Microbiology* **2**, 94.

For Review Only

638 Gschwendtner S, Leberecht M, Engel M, *et al.* (2015) Effects of elevated atmospheric CO_2 on microbial
639 community structure at the plant-soil interface of young beech trees (Fagus sylvatica L.) grown at two sites with
640 contrasting climatic conditions. *Microbial Ecology* **69**, 867-878.

641 Guelland K, Hagedorn F, Smittenberg R, *et al.* (2013) Evolution of carbon fluxes during initial soil formation
642 along the forefield of Damma glacier, Switzerland. *Biogeochemistry* **113**, 545-561.

643 Hagedorn F, Hiltbrunner D, Streit K, *et al.* (2013) Nine years of CO 2 enrichment at the alpine treeline
644 stimulates soil respiration but does not alter soil microbial communities. *Soil Biology and Biochemistry* **57**, 390-
645 400.

646 Hanada S, Sekiguchi Y (2014) The phylum Gemmatimonadetes. In: *The Prokaryotes: Other Major Lineages of*
647 *Bacteria and The Archaea* (eds. Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F), pp. 677-681.
648 Springer Berlin Heidelberg, Berlin, Heidelberg.

649 Hartmann M, Howes CG, VanInsberghe D, *et al.* (2012) Significant and persistent impact of timber harvesting
650 on soil microbial communities in Northern coniferous forests. *The ISME Journal* **6**, 2199-2218.

651 Hartmann M, Niklaus PA, Zimmermann S, *et al.* (2014) Resistance and resilience of the forest soil microbiome
652 to logging-associated compaction. *The ISME Journal* **8**, 226-244.

653 Herzog C, Steffen J, Graf Pannatier E, Hajdas I, Brunner I (2014) Nine years of irrigation cause vegetation and
654 fine root shifts in a water-limited pine forest. *PLoS ONE* **9**, e96321.

655 Hibbett DS, Bauer R, Binder M, *et al.* (2014) Agaricomycetes. In: *The Mycota VII: Systematics and Evolution*
656 *Part A* (eds. McLaughlin JD, Spatafora WJ), pp. 373-429. Springer Berlin Heidelberg, Berlin, Heidelberg.

657 Hibbett DS, Binder M, Bischoff JF, *et al.* (2007) A higher-level phylogenetic classification of the Fungi.
658 *Mycological Research* **111**, 509-547.

659 Hsieh T, Ma K, Chao A (2013) iNEXT: iNterpolation and EXTrapolation for species diversity. *R package*
660 *version 2.0.8*, 1-18.

661 Kersters K, De Vos P, Gillis M, Swings J (2001) Proteobacteria. In: *Encyclopedia of Life Sciences*, p. 13. John
662 Wiley & Sons, Ltd.

663 Kielak AM, Barreto CC, Kowalchuk GA, Van Veen JA, Kuramae EE (2016) The ecology of Acidobacteria:
664 moving beyond genes and genomes. *Frontiers in Microbiology* **7**.

665 Knapp DG, Pintye A, Kovács GM (2012) The dark side Is not fastidious – dark septate endophytic fungi of
666 native and invasive plants of semiarid sandy areas. *PLoS ONE* **7**, e32570.

667 Koyama A, Wallenstein MD, Simpson RT, Moore JC (2014) Soil bacterial community composition altered by
668 increased nutrient availability in Arctic tundra soils. *Frontiers in Microbiology* **5**.

669 Lee KC, Dunfield PF, Stott MB (2014) The phylum Armatimonadetes. In: *The Prokaryotes: Other Major*
670 *Lineages of Bacteria and The Archaea* (eds. Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F),
671 pp. 447-458. Springer Berlin Heidelberg, Berlin, Heidelberg.

672 Lee Taylor D, Sinsabaugh RL (2015) The soil fungi: occurrence, phylogeny, and ecology. In: *Soil Microbiology,*
673 *Ecology and Biochemistry* (ed. Paul EA), pp. 77-109. Academic Press, Boston, USA.

674 Leuzinger S, Luo Y, Beier C, *et al.* (2011) Do global change experiments overestimate impacts on terrestrial
675 ecosystems? *Trends in Ecology & Evolution* **26**, 236-241.

676 Li H, Xu Z, Yang S, *et al.* (2016) Responses of soil bacterial communities to nitrogen deposition and
677 precipitation increment are closely linked with aboveground community variation. *Microbial Ecology* **71**, 974-
678 989.

For Review Only

679 Lindahl BD, Ihrmark K, Boberg J, *et al.* (2007) Spatial separation of litter decomposition and mycorrhizal
680 nitrogen uptake in a boreal forest. *New Phytologist* **173**, 611-620.

681 Lladó S, Žifčáková L, Větrovský T, Eichlerová I, Baldrian P (2016) Functional screening of abundant bacteria
682 from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide
683 decomposition. *Biology and Fertility of Soils* **52**, 251-260.

684 Manzoni S, Schimel JP, Porporato A (2012) Responses of soil microbial communities to water stress: results
685 from a meta-analysis. *Ecology* **93**, 930-938.

686 Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *The*
687 *ISME Journal* **7**, 830-838.

688 McBride MJ, Liu W, Lu X, Zhu Y, Zhang W (2014) The family Cytophagaceae. In: *The Prokaryotes: Other*
689 *Major Lineages of Bacteria and The Archaea* (eds. Rosenberg E, DeLong EF, Lory S, Stackebrandt E,
690 Thompson F), pp. 577-593. Springer Berlin Heidelberg, Berlin, Heidelberg.

691 Mohammadipanah F, Wink J (2015) Actinobacteria from arid and desert habitats: diversity and biological
692 activity. *Frontiers in Microbiology* **6**, 1541.

693 Naether A, Foesel BU, Naegele V, *et al.* (2012) Environmental factors affect acidobacterial communities below
694 the subgroup level in grassland and forest soils. *Applied and Environmental Microbiology* **78**, 7398-7406.

695 Navarrete AA, Venturini AM, Meyer KM, *et al.* (2015) Differential response of Acidobacteria subgroups to
696 forest-to-pasture conversion and their biogeographic patterns in the Western Brazilian Amazon. *Frontiers in*
697 *Microbiology* **6**, 1443.

698 Nguyen NH, Song Z, Bates ST, *et al.* (2016) FUNGuild: An open annotation tool for parsing fungal community
699 datasets by ecological guild. *Fungal Ecology* **20**, 241-248.

700 Nielsen UN, Ball BA (2015) Impacts of altered precipitation regimes on soil communities and biogeochemistry
701 in arid and semi-arid ecosystems. *Global Change Biology* **21**, 1407-1421.

702 Oksanen J, Blanchet FG, Kindt R, *et al.* (2016) vegan: Community Ecology Package (v2.3-3). *R package*
703 *version*, 1-17.

704 Pailler A, Vennetier M, Torre F, Ripert C, Guiral D (2014) Forest soil microbial functional patterns and response
705 to a drought and warming event: Key role of climate–plant–soil interactions at a regional scale. *Soil Biology and*
706 *Biochemistry* **70**, 1-4.

707 Pascault N, Ranjard L, Kaisermann A, *et al.* (2013) Stimulation of different functional groups of bacteria by
708 various plant residues as a driver of soil priming effect. *Ecosystems* **16**, 810-822.

709 Pašić L, Kovče B, Sket B, Herzog-Velikonja B (2010) Diversity of microbial communities colonizing the walls
710 of a Karstic cave in Slovenia. *FEMS Microbiology Ecology* **71**, 50-60.

711 Philippot L, Andersson SGE, Battin TJ, *et al.* (2010) The ecological coherence of high bacterial taxonomic
712 ranks. *Nature Reviews Microbiology* **8**, 523-529.

713 Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial
714 ecology of the rhizosphere. *Nature Reviews Microbiology* **11**, 789-799.

715 Poulter B, Frank D, Ciais P, *et al.* (2014) Contribution of semi-arid ecosystems to interannual variability of the
716 global carbon cycle. *Nature* **509**, 600-603.

717 R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical
718 Computing, Vienna, Austria (http://www.R-project.org/).

719 Rebetez M, Dobbertin M (2004) Climate change may already threaten Scots pine stands in the Swiss Alps.
720 *Theoretical and Applied Climatology* **79**, 1-9.

For Review Only

721 Reichstein M, Bahn M, Ciais P*, et al.* (2013) Climate extremes and the carbon cycle. *Nature* **500**, 287-295.

722 Richardson M (2009) The ecology of the Zygomycetes and its impact on environmental exposure. *Clinical*
723 *Microbiology and Infection* **15, Supplement 5**, 2-9.

724 Rigling A, Bigler C, Eilmann B*, et al.* (2013) Driving factors of a vegetation shift from Scots pine to pubescent
725 oak in dry Alpine forests. *Global Change Biology* **19**, 229-240.

726 Rime T, Hartmann M, Brunner I*, et al.* (2015) Vertical distribution of the soil microbiota along a successional
727 gradient in a glacier forefield. *Molecular Ecology* **24**, 1091-1108.

728 Rime T, Hartmann M, Frey B (2016) Potential sources of microbial colonizers in an initial soil ecosystem after
729 retreat of an alpine glacier. *The ISME Journal*.

730 Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (2014) *The Prokaryotes: Actinobacteria*
731 Springer Berlin Heidelberg, Berlin, Heidelberg.

732 Rosling A, Landeweert R, Lindahl BD*, et al.* (2003) Vertical distribution of ectomycorrhizal fungal taxa in a
733 podzol soil profile. *New Phytologist* **159**, 775-783.

734 Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for
735 ecosystem function. *Ecology* **88**, 1386-1394.

736 Schloss PD, Westcott SL, Ryabin T*, et al.* (2009) Introducing mothur: open-source, platform-independent,
737 community-supported software for describing and comparing microbial communities. *Applied and*
738 *Environmental Microbiology* **75**, 7537-7541.

739 Seneviratne SI, Nicholls N, Easterling D*, et al.* (2012) Changes in climate extremes and their impacts on the
740 natural physical environment. In: *Managing the risks of extreme events and disasters to advance climate change*
741 *adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change.*
742 (ed. Field CB), pp. 109-230. Cambridge University Press.

743 Shannon P, Markiel A, Ozier O*, et al.* (2003) Cytoscape: a software environment for integrated models of
744 biomolecular interaction networks. *Genome Research* **13**, 2498-2504.

745 Smit E, Leeflang P, Gommans S*, et al.* (2001) Diversity and seasonal fluctuations of the dominant members of
746 the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Applied and*
747 *Environmental Microbiology* **67**, 2284-2291.

748 Spatafora JW, Chang Y, Benny GL*, et al.* (2016) A phylum-level phylogenetic classification of zygomycete
749 fungi based on genome-scale data. *Mycologia* **108**, 1028-1046.

750 Storey J, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National*
751 *Academy of Sciences of the United States of America* **100**, 9440 - 9445.

752 Storey JD, Bass AJ, Dabney A, Robinson D (2015) qvalue: Q-value estimation for false discovery rate control. R
753 package version 2.2.2.

754 Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilisation in forest litter and soil:
755 identification of bacterial and fungal decomposers. *FEMS Microbiology Ecology* **80**, 735-746.

756 Suh S-O, Blackwell M, Kurtzman CP, Lachance M-A (2006) Phylogenetics of Saccharomycetales, the
757 ascomycete yeasts. *Mycologia* **98**, 1006-1017.

758 Vacchiano G, Garbarino M, Mondino EB, Motta R (2012) Evidences of drought stress as a predisposing factor
759 to Scots pine decline in Valle d'Aosta (Italy). *European Journal of Forest Research* **131**, 989-1000.

760 van der Molen MK, Dolman AJ, Ciais P*, et al.* (2011) Drought and ecosystem carbon cycling. *Agricultural and*
761 *Forest Meteorology* **151**, 765-773.

For Review Only

762 Ventura M, Canchaya C, Tauch A, *et al.* (2007) Genomics of Actinobacteria: Tracing the Evolutionary History
763 of an Ancient Phylum. *Microbiology and Molecular Biology Reviews* **71**, 495-548.

764 von Rein I, Gessler A, Premke K, *et al.* (2016) Forest understory plant and soil microbial response to an
765 experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. *Global*
766 *Change Biology* **22**, 2861-2874.

767 Voriskova J, Baldrian P (2013) Fungal community on decomposing leaf litter undergoes rapid successional
768 changes. *The ISME Journal* **7**, 477-486.

769 Voříšková J, Brabcová V, Cajthaml T, Baldrian P (2014) Seasonal dynamics of fungal communities in a
770 temperate oak forest soil. *New Phytologist* **201**, 269-278.

771 Wang X, Sharp CE, Jones GM, *et al.* (2015) Stable-isotope probing identifies uncultured planctomycetes as
772 primary degraders of a complex heteropolysaccharide in soil. *Applied and Environmental Microbiology* **81**,
773 4607-4615.

774 Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS (2006) Toward a phylogenetic classification of
775 the Leotiomycetes based on rDNA data. *Mycologia* **98**, 1065-1075.

776 Waring BG, Hawkes CV (2015) Short-term precipitation exclusion alters microbial responses to soil moisture in
777 a wet tropical forest. *Microbial Ecology* **69**, 843-854.

778 Wolf AB, Vos M, de Boer W, Kowalchuk GA (2014) Impact of Matric Potential and Pore Size Distribution on
779 Growth Dynamics of Filamentous and Non-Filamentous Soil Bacteria. *PLoS ONE* **8**, e83661.

780 Youssef NH, Farag IF, Rinke C, *et al.* (2015) In silico analysis of the metabolic potential and niche
781 specialization of candidate phylum "Latescibacteria" (WS3). *PLoS ONE* **10**, e0127499.

782 Zeng Q, Dong Y, An S (2016) Bacterial community responses to soils along a latitudinal and vegetation gradient
783 on the Loess Plateau, China. *PLoS ONE* **11**, e0152894.

784 Zhao M, Running SW (2010) Drought-induced reduction in global terrestrial net primary production from 2000
785 through 2009. *Science* **329**, 940-943.

786 Zhou J, Gu Y, Zou C, Mo M (2007) Phylogenetic diversity of bacteria in an earth-cave in Guizhou Province,
787 Southwest of China. *The Journal of Microbiology* **45**, 105.

788 Zogg GP, Zak DR, Ringelberg DB, *et al.* (1997) Compositional and functional shifts in microbial communities
789 due to soil warming. *Soil Science Society of America Journal* **61**, 475-481.
790

791

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792 **Figure legends**

793

794 **Figure 1**. Irrigation effects on bacterial and fungal diversity along the soil depth profile. Changes in
795 β-diversity were assessed by analysis of principal coordinates (PCO) based on Bray-Curtis
796 similarities calculated from relative OTU abundances. PCO ordinations were generated separately for
797 bacteria (B) and fungi (F), and overlaid using procrustes analysis (bacterial and fungal data points
798 from the same sample are connected by lines; procrustes goodness of fit m2=0.09, $P<0.001$; mantel
799 test of underlying similarity matrices r=0.81, $P<0.001$). The 99% confidence ellipses for the centroids
800 of each cluster are provided for bacteria and fungi separately. The variance explained by each PCO
801 axis is given in parentheses (first for bacteria, second for fungi). Changes in α-diversity were
802 assessed by calculating Shannon diversity indices based on iteratively rarefied OTU counts and
803 visualized by boxplots (bacteria: upper panel; fungi: lower panel) including the individual values.
804 Effect strengths were assessed by permutational analysis of variance (PERMANOVA) and are
805 provided in Table 2. Org: organic F-horizon; Min 2: mineral soil (0 – 2 cm depth); Min 10: mineral
806 soil (5 – 10 cm depth).

807

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

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819 significance (nodes with $q \geq 0.15$ are completely grey and nodes with $q \leq 0.05$ are completely
820 red/blue).
821
822 **Figure 3**. Relative change (z-transformed) in abundance of all bacterial and fungal phyla (upper panel)
823 as well as the major proteobacterial, ascomycetous and basidiomycetous classes (lower panel). The
824 first vertical panel represents the relative change in abundance from the overall mean, including the
825 average change (vertical lines) and the corresponding standard error (boxes, n=4). The second vertical
826 panel shows the strength of the irrigation effect as assessed by permutational analysis of variance
827 (PERMANOVA) including the F-ratio (visualized by differently sized circles) as well as the
828 uncorrected (p) and corrected (q) levels of significance. The third vertical panel shows the degree of
829 depth-dependency of the irrigation effect (irrigation × depth interaction) as assessed by
830 PERMANOVA including the F-ratio (visualized by differently sized circles) as well as the
831 uncorrected (p) and corrected (q) levels of significance. The fourth vertical panel shows the relative
832 abundance (percent reads standardized by domain) as well as the number of OTUs for each
833 investigated taxon, both metrics being visualized by differently sized circles.
834
835 **Supplementary Figure 1**. (a) Monthly mean volumetric water content (%) of the irrigated (dashed
836 blue) and the control (red) plots over the experimental period (2003 – 2013). Annual precipitation
837 (mm) and the applied annual irrigation (b). Irrigation periods are indicated as grey bars. The figure
838 was adapted from Herzog *et al.* (2014).
839
840 **Supplementary Figure 2**. Rarefaction (solid lines) and extrapolation (dashed lines) curves of the
841 observed bacterial and fungal OTU richness at different soil depths in the irrigated and dry plots.
842 Samples from the same treatment and soil horizon were pooled prior to analysis. The solid circles
843 represent the actually observed value, whereas the shaded regions represent the 95% confidence
844 intervals obtained by bootstrapping with 200 replications. Org: organic F-horizon; Min 2: mineral soil
845 (0 – 2 cm depth); Min 10: mineral soil (5 – 10 cm depth).
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847 **Supplementary Figure 3**. Relative (standardized and scaled) change in abundance of all bacterial and
848 fungal phyla (upper panel) as well as the major proteobacterial, ascomycetous and basidiomycetous
849 classes (lower panel). These results are equivalent to Figure 2, but only based on the mineral soil
850 (excluding the organic horizon). See legend of Figure 2 for further details.
851
852 **Supplementary Figure 4**. Relative (standardized and scaled) change in abundance of all bacterial and
853 fungal taxa from phylum to genus level. These results are equivalent to Figure 2 and provide the
854 complete corresponding statistics for all detected phyla, classes, orders, families and genera. The taxa
855 are ordered hierarchically with lower taxonomic levels showing increasingly stronger indentation.
856 Taxon labels (green for bacteria and red for fungi) of different phyla are separated by horizontal lines.
857 See legend of Figure 2 for further details.
858
859 **Supplementary Figure 5**. Relative (standardized and scaled) change in abundance of all bacterial and
860 fungal taxa from phylum to genus level. These results are equivalent to Supplementary Figure 4, but
861 only based on the mineral soil (excluding the organic horizon). See legend of Supplementary Figure 4
862 for further details.

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Table 1. Abiotic and biotic site characteristics of dry and irrigated plots of the Pfynwald 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.

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

	Bacteria		Fungi	
All horizons	α-diversity[1]	β-diversity[1]	α-diversity	β-diversity
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 × 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 × 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).

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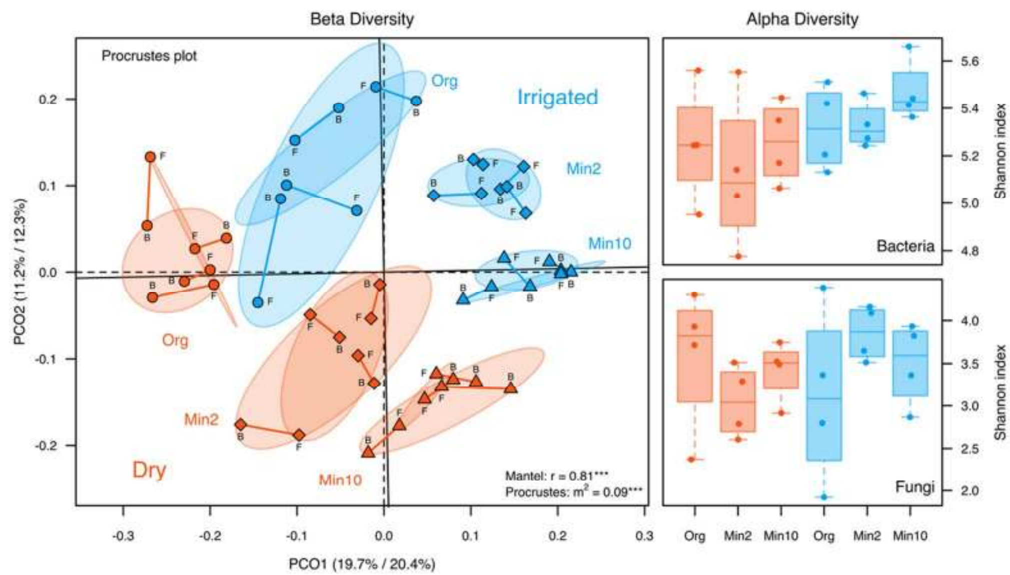

Figure 1

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View Only

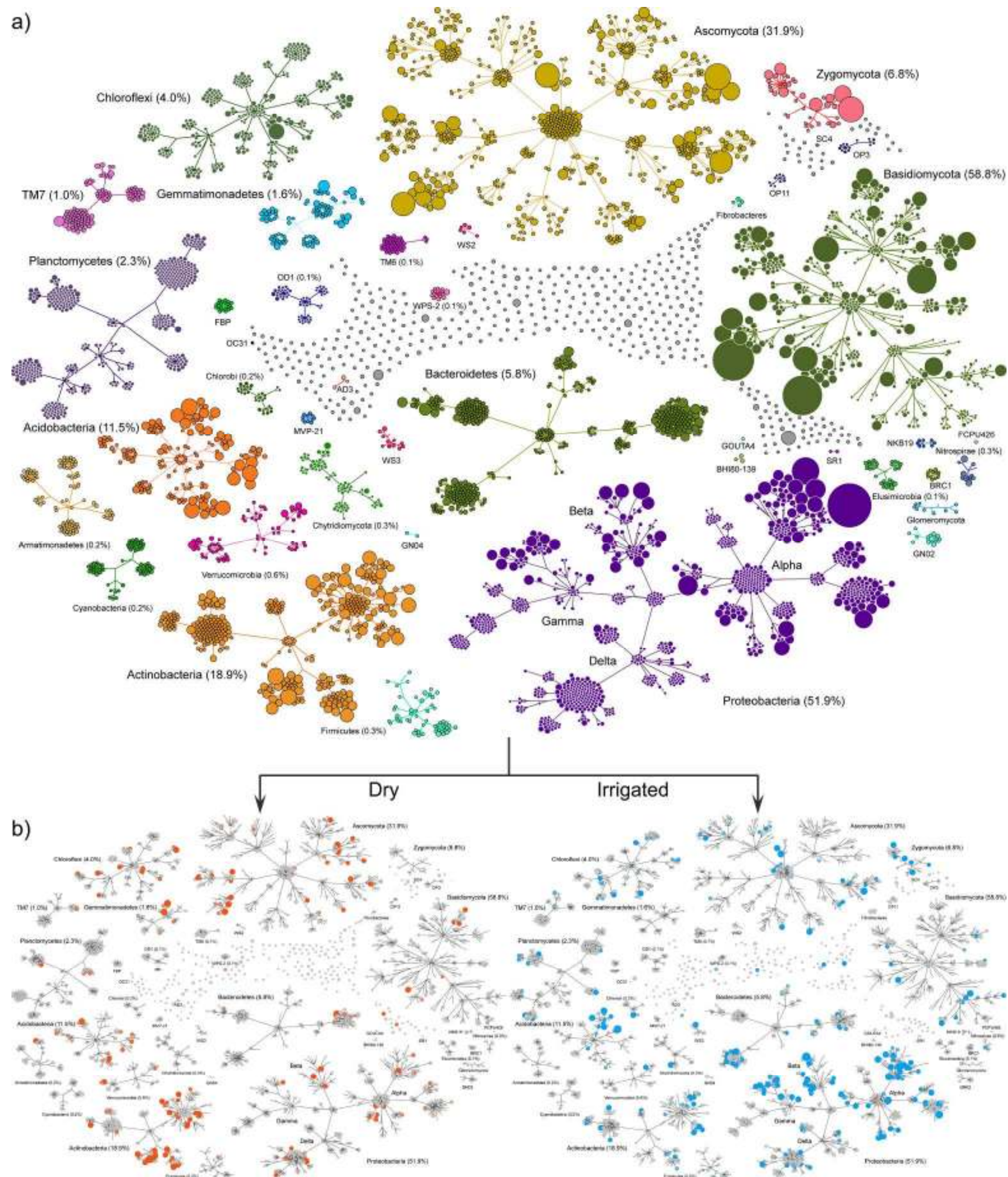

Figure 2

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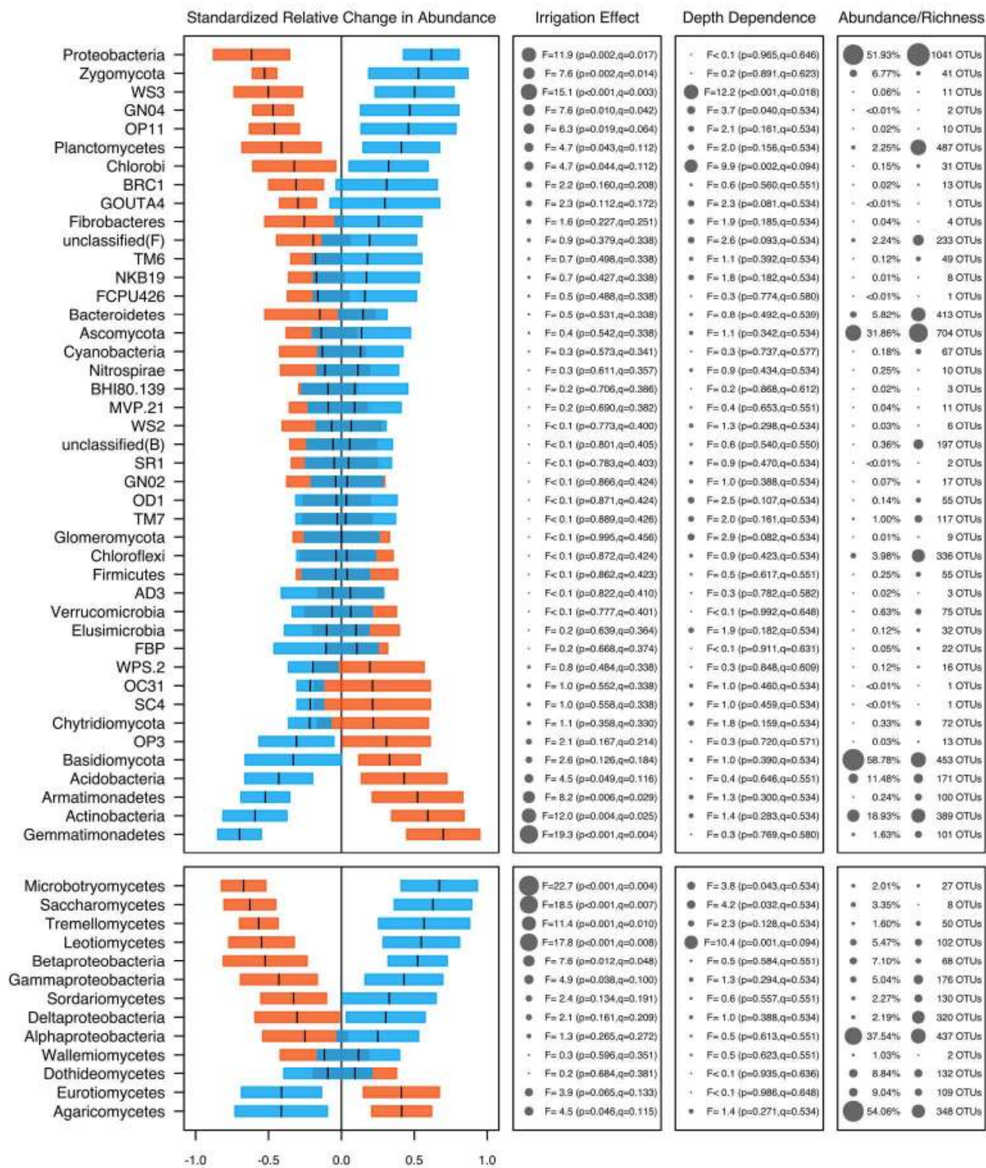

Figure 3

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