1   **Extreme drought triggers transition to an alternative soil microbial state**

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11 SUMMARY

12   Soil microbial communities play a pivotal role in regulating ecosystem
 13 functioning¹ but they are increasingly threatened by human-driven perturbations,
 14 including climate extremes, which are predicted to increase in frequency and intensity
 15 with climate change². It has been demonstrated that soil microbial communities are
 16 sensitive to climate extremes, such as drought^{3,4}, and that effects can be long-lasting^{5,6}.
 17 However, considerable uncertainties remain concerning the response of soil microbial
 18 communities to increases in the intensity and frequency of climate extremes, and their
 19 potential to trigger transitions to alternative, and potentially deleterious, taxonomic and
 20 functional states⁷. Here we demonstrate that extreme, frequent drought induces a shift to
 21 an alternative soil microbial state characterised by strongly altered bacterial and fungal
 22 community structure of reduced complexity and functionality. Moreover, we found that
 23 this drought-induced alternative microbial state persisted after returning soil to its
 24 previous moisture status. However, bacterial communities were able to adapt by
 25 increasing their growth capacity, despite being of reduced diversity. Abrupt transitions
 26 to alternative states are well documented in aquatic and terrestrial plant communities in
 27 response to human-induced perturbations, including climate extremes^{8,9}. Our results
 28 provide experimental evidence that such transitions also occur in soil microbial
 29 communities in response to extreme drought with potentially deleterious consequences
 30 for soil health.

31

32 MAIN

33   Natural ecosystems are constantly exposed to natural fluctuations in environmental
 34 conditions and under such conditions they retain a stable equilibrium state, or quasi-

35 stable state, characterized by minor fluctuations in community composition and
 36 function¹⁰. However, human-induced perturbations, such as those related to climate
 37 change, can destabilise this dynamic equilibrium and potentially trigger a cascade of
 38 events that may lead to an abrupt change from one ecosystem state to another⁸. Abrupt
 39 transitions occur when an ecosystem moves from one attraction basin into a different
 40 one in its stability landscape after surpassing a certain threshold⁸. This can have
 41 important consequences for ecosystem functioning, as the ecosystem stays in the new
 42 attraction valley – the alternative state – even if the perturbation that triggered the
 43 change has ceased. Numerous studies have demonstrated the existence of abrupt
 44 changes to alternative states in aquatic ecosystems^{9,11}, terrestrial plant communities^{12,13},
 45 and the human gut microbiome in response to perturbations^{14,15}. However, abrupt
 46 transitions to alternative states in soil microbial communities have so far received little
 47 attention⁷, despite their fundamental role in terrestrial ecosystems, driving key processes
 48 of organic matter decomposition, nutrient cycling, and carbon and nutrient storage¹⁶,
 49 which regulate ecosystem productivity. As such, abrupt transitions in soil microbial
 50 communities from one state to another may have significant implications for soil
 51 functioning, with consequences for ecosystem services, such as food production and
 52 climate regulation¹.

53 Soil microbial communities are increasingly challenged by perturbations associated
 54 with human-induced environment change, including intense ‘pulse’ perturbations¹⁷
 55 caused by climate extremes (e.g. droughts, heat waves and floods), which are predicted
 56 to increase in frequency and intensity as a consequence of ongoing climate change².
 57 Previous studies have shown that soil microbial communities are sensitive to drought,
 58 with different microbial groups varying in their tolerance and ability to recover from
 59 drought^{3,18,19}. Moreover, extreme drought has been shown to de-stabilize soil bacterial

60 networks, potentially rendering them more vulnerable to subsequent drought²⁰, although
 61 repeated droughts can also lead to soil microbial communities becoming more resistant
 62 to drought^{5,21,22}. Extreme drought has been shown to shift peatland moisture
 63 characteristics²³ and plant-soil fungi interactions²⁴ to an alternative state, and repeated
 64 dry-wet cycles induced shifts in the functional state of agricultural soils, measured as
 65 soil respiration²⁵. However, evidence of transitions to alternative states in soil microbial
 66 communities in response to perturbations, such as drought, is still lacking. Specifically,
 67 it remains unresolved whether increases in the intensity and frequency of drought has
 68 potential to trigger an abrupt change in soil microbial communities to an alternative
 69 taxonomic and functional microbial state.

70 Here, we experimentally tested whether increases in the intensity and frequency of
 71 drought triggers a shift to an alternative, functionally deleterious state in soil microbial
 72 communities. Petraitis and Dugeon²⁶ proposed that experimental evidence of the
 73 existence of alternative stable states must fulfil three conditions: it must (a) demonstrate
 74 that communities differ significantly in their assembly and functional attributes within
 75 the same environment; (b) vary with the scale of the pulse perturbation, given that small
 76 perturbations are likely to be insufficient to tip the system from one state to another,
 77 whereas large perturbations are more likely to do so; and (c) be carried out over a
 78 sufficient time period to ensure that the alternative states are self-sustaining or stable.
 79 To meet these conditions, and experimentally test for the existence of alternative states
 80 in soil microbial communities, we carried out an incubation experiment in a plant-free
 81 system whereby we imposed a matrix of drought frequency and intensity treatments
 82 (one, two, three drought events and mild, intermediate and extreme drought,
 83 respectively), followed by the assessment over an extended period of time of a broad
 84 range of taxonomic and functional attributes of microbial communities in a natural

85 grassland soil. The experimental system, although simplified, allowed us to provide
86 experimental proof of concept of the existence of alternative states in soil microbial
87 communities. We discovered that high intensity drought triggered a shift to an
88 alternative microbial state of reduced structural complexity and impaired functioning.
89 We also discovered that the functional characteristics of soil microbial communities
90 could adapt to drought exposure, revealing a faster ability of bacterial communities to
91 recover growth rates following drought in the alternative state.

92 **Changes in microbial community structure**

93 Our findings show that extreme drought (~11% WHC), simulating a once in a
94 century drought in England (see methods), had a profound and long-lasting impact on
95 soil microbial community structure and diversity, despite environmental conditions
96 being returned to their original state (optimum moisture, 65% WHC). Six months after
97 the end of the extreme drought, bacterial diversity (Fig 1a, b) was still reduced, while
98 fungal Shannon diversity was significantly higher than in the non-droughted control
99 treatment (Fig. 1d), despite no effects on fungal species richness being detected (Fig.
100 1c). These contrasting responses (reduced bacterial diversity but increased fungal one)
101 are consistent with previous findings^{20,27}. Bacterial and fungal community structure was
102 strongly affected by drought (Fig. 1e, f, Extended Data Fig.1a, b) and changes in
103 microbial community structure observed after drought were exacerbated with time. This
104 is exemplified in the proportion of variance in community structure explained by
105 drought intensity (Fig. 1g), which increased after 6 months compared to immediately
106 after drought, particularly under the extreme drought treatment. However, bacterial
107 communities in soils subjected to intermediate (23% WHC) drought, which simulated
108 drought events in England occurring every 4 years, partially recovered (Fig. 1g), with
109 less variance explained by drought treatments after 6 months than after drought, i.e.,

they were more similar to the control treatments after 6 months than at the end of the perturbation. Mild drought (40% WHC), which simulated common drought events occurring annually in England, had no detectable impact on microbial community structure. Drought frequency also significantly affected bacterial and fungal community structure (Fig. 1e, f), with those soils exposed to more frequent drought being more distinct from the non-droughted control and soil subject to fewer drought events.

Sixty-eight of the 82 indicator genera identified for bacteria (Extended Data Fig. 2a) were indicators for all of the groups except the extreme drought, i.e. their abundance was significantly reduced under extreme drought. The proportion of different bacterial taxa observed in the soil communities was mainly affected by drought intensity and time, with some minor effects of drought frequency (Extended Data Table 1). Bacterial phyla Proteobacteria, Actinobacteria and Firmicutes increased in relative abundance with drought intensity, while Acidobacteria and Bacteroidetes decreased (Extended Data Fig. 1c). There was a clear shift in the bacterial community, persistent through time, from a community co-dominated by Acidobacteria and Proteobacteria in the control towards one dominated by Proteobacteria under extreme drought. Some of these Phyla-level responses were consistent with previous reports. Actinobacteria^{18,21} and Firmicutes^{28,29} have been frequently reported as drought tolerant. However, there are contrasting references to Proteobacteria, with some studies defining them as tolerant to drought⁵, and others showing a decrease in their abundance after drought²⁷. On the other hand, Acidobacteria¹⁸ and Bacteroidetes²⁷ have been described as sensitive to drought, as found in this study, although there are also some contradictory reports in the literature for both phyla^{5,30}.

At family level, particularly significant were the increases in Xanthobacteraceae, Chthoniobacteraceae, Rhodanobacteraceae and a transient increase in Burkholderiaceae

135 after drought (Fig. 1h), and the decrease in the relative abundance of Solibacteraceae
 136 and Pedosphaeraceae with drought intensity (Fig. 1h). An increase in
 137 Xanthobacteraceae in soils under drought stress has been previously reported³¹. Within
 138 the family Chthoniobacteraceae, the most abundant genus was *Candidatus*
 139 *Udaeobacter*. This is an extremely abundant genus in soils, adapted to grow efficiently
 140 under limited resources³², which could be the case in the drought exposed soils of this
 141 experiment.. The Rhodanobacteraceae family was dominated by the genus
 142 *Rhodanobacter*. This genus is common in soils and some species have been identified
 143 as plant growth promoting rhizobacteria with drought protection capacities³³. Finally,
 144 the transient increase in Burkholderiaceae after drought was mostly driven by an
 145 increase in the genus *Massilia*. They have been identified as copiotrophic bacteria that
 146 can quickly grow under high nutrient conditions (such as those created at rewetting) but
 147 that lose their competitive advantage later on³⁴, which can explain the pattern observed
 148 in this experiment. Regarding those families that decreased with drought, both agree
 149 with the literature, as Solibacteraceae¹⁸ and Pedosphaeraceae³⁵ have been found to
 150 decrease with decreasing soil moisture.

151 Fungal indicator genera (Extended Data Fig. 2b) were mostly identified for the
 152 control + mild + intermediate drought group (17 out of 20), with no genus indicator for
 153 the extreme drought. The relative abundance of main fungal phyla was mostly affected
 154 by drought intensity and time (Extended Data Fig. 1e, Extended Data Table 1). We
 155 observed an increase in Ascomycota and a decrease in Mortierellomycota. In particular,
 156 we observed an increase in relative abundance of Piskurozymaceae, Helotiales,
 157 Trimorphomycetaceae and Nectriaceae, and a substantial decrease of Mortierellaceae
 158 and Clavariaceae, the two most abundant fungal families (Fig. 1i), which are both
 159 typical soil saprotrophs. In agreement with this, we observed a significant decrease with

drought treatments in the relative abundance of taxa considered as saprotrophs (Extended Data Fig. 1g). Therefore, the observed increase in fungal diversity (Shannon index, Fig. 1d) was not associated with a change in species richness, but with an increase in evenness, as a result of decreasing abundances of the two major fungal taxa, Mortierellaceae and Clavariaceae. These two families have been previously identified as drought sensitive^{20,36}. The observed effects could be related to the changes in nutrient availability elicited by drought (see section below). Fungi are generally considered to be more resistant to drought than bacteria^{20,37} and several studies demonstrate a lack of drought effect on fungal communities³. In contrast, we observed a clear change in the fungal communities, which was still evident 6 months after returning droughted soils to their original, pre-drought moisture content.

Bacterial diversity is strongly affected by soil pH at a global scale³⁸ and at local scales³⁹, while its impact on fungal diversity is weak or absent. In our experiment, bacterial diversity and community structure were correlated with a decrease in soil pH at the last sampling point (Extended Data Fig. 3a-c), associated with increased nitrate ion concentrations at this sampling time (Extended Data Fig. 3f). In agreement with the literature^{39,40}, fungal diversity was only marginally affected by soil pH (Extended Data Fig. 3d-e).

Changes in microbial community function

Our data show that microbial community shifts in response to extreme drought were associated with persistent changes in microbial functioning (Extended Data Fig. 3g-h). Soils exposed to extreme drought were in a significantly different functional state than the control soils even 6 months after the end of the perturbation (Fig. 2c, d).

183 After the end of drought, when soils were rewetted to the level of the control, seven
 184 of the eight extracellular enzyme activities evaluated were significantly reduced by
 185 drought intensity and/or frequency, displaying a low resistance to drought (Fig. 2a,
 186 Extended Data Fig. 4a-h, Extended Data Table 2). Phenoloxidase (POX) activity was
 187 slightly reduced by all drought treatments compared to the control, with no significant
 188 effect of drought intensity or frequency levels (Fig. 2a, Extended Data Fig. 4g). Only
 189 the activity of peroxidase (PER) was not affected by drought (only a marginal effect of
 190 drought frequency) (Extended Data Fig. 4h). The effects of drought intensity on soil
 191 enzymes were still detectable after six months (Extended Data Fig. 4i-p), with very little
 192 or no resilience, or even a stronger reduction of their activity than immediately after
 193 drought (phosphatase) (Fig. 2b), manifesting a persistent reduction of soil functional
 194 capacity; a functional regime shift. A reduction in soil enzymatic capacity with drought
 195 has been frequently reported⁴¹, probably associated with microbial death and thus
 196 reduced enzyme production, and reduced substrate diffusion that limits enzyme
 197 activity⁴². However, these effects are quite context dependent⁴¹ and some authors have
 198 reported increased enzyme activities under drought^{43,44}, which could be related to a
 199 reduced degradation rate of soil enzymes and accumulation during dry periods⁴². In our
 200 experiment, enzyme activities correlated with available nutrients (Extended Data Fig.
 201 3i-k). Additionally, consistently low microbial biomass after 6 months (Fig. 2b,
 202 Extended Data Fig. 3l) could explain the lack of recovery of enzymatic capacities.
 203 Ligninases (POX and PER) were less affected by drought than the hydrolytic enzymes,
 204 which could be related to the fact that fungal communities were less affected by drought
 205 than bacterial ones, as ligninases are mostly produced by fungi⁴².

206 Soil nutrients were highly affected by drought, particularly available ammonium and
 207 DOC, with a significant increase due to drought intensity and frequency and an

208 interaction between them: the more intense the drought, the bigger the effect of drought
 209 frequency on their concentration (Fig. 2a, Extended Data Fig. 5a-f, Extended Data Table
 210 2). Increases in nutrient availability after rewetting dry soils have been frequently
 211 explained by the death of microorganisms and the fact that some organic compounds in
 212 the soil become more available after drought^{45,46}. On the other hand, soil nitrate
 213 concentrations were reduced with most drought treatments compared to the control,
 214 showing a low resistance (Fig. 2a). After six months, this big ammonium and DOC
 215 flush had mostly disappeared and in turn, nitrate levels significantly increased (Fig. 2b,
 216 Extended Data Fig. 5k-p). This could be related to a high nitrification activity, where
 217 the highly abundant ammonium was transformed into nitrate⁴⁷.

218 Microbial biomass C, N and P were reduced by increasing drought intensity, and this
 219 effect was still detected after 6 months of returning soils to moisture levels of the
 220 control, with microbial P in extreme drought treatments showing a stronger decrease
 221 with time (Fig 2b, Extended Data Fig. 5g-i, q-s, Extended Data Table 2). Decreases in
 222 microbial biomass due to drought have been widely reported^{27,48}, although some authors
 223 observed an increase in microbial biomass under drought⁴⁹. The constant decline of
 224 microbial P over time could be related to reduced phosphatase activity (Extended Data
 225 Fig. 3m). This is in agreement with the study of Dijkstra and collaborators⁵⁰ that
 226 showed a strong reduction in microbial P uptake under drought. However, a decrease in
 227 microbial biomass over time in bare soils is expected, as there is no additional C input
 228 from plants into the system.

229 **Adaptation of microbial growth characteristics to drought**

230 Our assessment of the responses of microbial communities with a history of drought
 231 to a further drying/rewetting cycle helped us examine legacy effects of drought and

adaptation of growth responses, as a community, of bacteria and fungi. Drought intensity had a legacy effect resulting in shorter lag times and higher cumulative growth (Fig. 3a, b) after rewetting, indicating a faster recolonization ability. The increased and faster bacterial growth in soils with a drought history has been previously observed²², and can be a good adaptive strategy in soils exposed to frequent drought events. On the other hand, cumulative fungal growth (Fig. 3c) significantly decreased with previous drought intensity, which could underpin the reduced abundance of the two dominant fungal families. This result contrasts with other studies where fungal growth was not affected by drought history^{19,51}. However, the fungal growth capacity in our study could have also been constrained by the high bacterial growth in the soils⁵². These effects on microbial growth were not dependent on the microbial biomass of soils before drying/rewetting (Extended Data Fig. 3n,o). Cumulative respiration after rewetting significantly decreased with previous drought intensity (Fig. 3d), and thus it seems to be driven by fungi, as it follows approximately the same pattern as cumulative fungal growth. This contrasts with some recent observations, where respiration was mostly driven by bacterial growth¹⁹. Alternatively, this decrease in cumulative respiration with previous drought intensity could be also related to resource availability. Previous extreme drought led to a strong increase of DOC, which was mostly used one month after drought, and this likely depleted the soil carbon available to fuel a respiration peak after the additional drying/rewetting cycle. Reduced carbon availability in soils after drought has been previously reported⁴⁷, as well as less intense respiration peaks after repeated drying/rewetting cycles^{22,47}.

Fungi were more resistant to low moisture than bacteria, as shown by the IC₁₀ (moisture level at which growth rate is reduced by 10%), but this was not significantly affected by previous drought treatments (Extended Data Fig. 6). However, there were

some trends evident, with bacteria and fungi responding in opposite directions. While previous exposure to drought had a tendency to reduce bacterial resistance to drought (higher IC values), fungi tended to show a higher resistance to drought under some of the previous treatments (lower IC values), although this was quite variable. Respiration showed reduced resistance to drought with previous drought intensity.

We observed legacy effects of drought on soil microbial communities, as previously described. For example, pre-exposure to drought has been demonstrated to increase bacterial resistance^{5,21} and resilience²² to subsequent droughts, although others have reported the opposite pattern, with previous drought damaging microbial communities in the long-term^{20,27}. We observed an increased recolonization capacity of bacteria, albeit a lower tolerance to reduced soil moisture, while fungi showed a potential increased resistance to drought but with less cumulative growth upon rewetting. Thus, there seems to be a trade-off between growth after rewetting, which can be interpreted as resilience, and resistance to low moisture, as previously proposed⁵³. These strategy changes could be the result of a shift in the relative abundance of different taxa within the community or due to changes in individual taxa's physiology or traits (evolution). In any case, the response of fast re-coloniser bacterial taxa appears to shape bacterial communities after drought, as they occupy empty niches left after drought, conditioning community assembly afterwards⁵⁴.

Drought threshold and shift to alternative state

Our findings provide experimental evidence that the harshness of drying determined the response pattern of microbes upon rewetting. Although gradient designs are best suited to evaluate thresholds⁵⁵, the design of the present experiment with four levels of soil moisture provides evidence of a potential threshold for a shift to an alternative

281 microbial state at a water content of ~15% WHC (~9% volumetric soil water content),
 282 which corresponds to a 30 year recurrent drought event in England (Extended Data Fig.
 283 6d-g). Below this moisture level, soil microbial communities were considerably and
 284 persistently restructured with impaired functioning, and they failed to recover over a
 285 period of 6 months, despite returning moisture levels to those of the control. The
 286 existence of this threshold is further supported by a previous study that demonstrated
 287 that below a threshold of 14% WHC, the growth pattern of bacteria upon rewetting
 288 changed significantly⁵⁶.

289 To further evaluate potential shifts to alternative states in our experiment, we mapped
 290 microbial community composition and function with multivariate analysis, as it is a
 291 useful tool to visualise stability landscapes and regime shifts⁵⁷. We observed a clear
 292 pattern in both community structure and function of soil microbial communities where
 293 soils subjected to extreme drought occupy a clearly distinct space separated from the
 294 control, which can be interpreted as an alternative basin in the stability landscape, an
 295 alternative state of the system (Extended Data Fig. 6h-k). Moreover, our experiment met
 296 most of the recognised criteria for detecting an alternative state²⁶. First, we
 297 demonstrated the existence of two different microbial communities given the same
 298 environmental condition (optimum soil moisture during the period after drought).
 299 Second, different intensities of drought (different scale of pulse perturbation) were
 300 found to have contrasting effects on soil microbial communities, with mild and
 301 intermediate drought showing reversible effects, but persistent changes in response to
 302 extreme drought. Third, our experiment was conducted over an extended period of time,
 303 representing the typical length of the growing season in the north of England, where the
 304 soil samples were collected. The identified microbial shifts are unlikely to represent a
 305 “stable” alternative state. Nevertheless, we experimentally demonstrate that microbial

communities subject to extreme drought shift towards a different state that is distinct from its original one and also from the non-droughted control soils. Therefore, we propose that the detected microbial shifts reflect an alternative “transient” state⁵⁴ or simply an alternative state.

Conclusion

Our findings experimentally demonstrate that extreme and frequent drought triggers a shift to an alternative microbial state, with pronounced and persistent reduction in soil microbial community functional capacity, modified taxonomical composition of reduced complexity, and faster ability of bacterial communities to recover growth rates following drought. Moreover, we show that this state transition was driven by drought intensity and occurred at a threshold of ~15% WHC, although more frequent and extreme events produced stronger effects than milder and less frequent drought events. Future studies are needed to consider the role of extrinsic factors that might modify the vulnerability of soil microbial communities to drought-induced transitions to alternative states, such as the presence of plants, which can help the system recover after drought⁵⁸, or differences in nutrient availability and other soil abiotic properties⁷. Nevertheless, our results provide experimental evidence for the existence of alternative taxonomic and functional states in soil microbial communities driven by extreme drought, with potentially deleterious consequences for soil health.

326 METHODS

327 Soil collection and experimental design

328 A pot experiment was designed to test the effects of drought intensity and frequency
 329 (3 levels each, full-factorial including a well-watered control), on the microbial
 330 communities in grassland soils. Soils were collected in Selside, Yorkshire Dales (54.17
 331 N, 2.34 W), from four independent plots (replicates). Turf was removed (2-3 cm), and
 332 topsoil collected from 3-10 cm depth. Main soil characteristics are shown in Extended
 333 Data Table 3. Soils were sieved (4 mm mesh) and divided into pots (8 cm height, 8 cm
 334 diameter, and 170 ml volume), filled to mimic the mean field bulk density (0.82 g cm^{-3}).
 335 Pots were incubated at 18 °C, 30% air relative humidity, and kept at 65% water holding
 336 capacity (WHC), which correspond to ~40% volumetric water content. After 3 weeks of
 337 stabilisation, drought treatments were applied by different watering regimes: control
 338 treatments received 100% of the water loss, mild drought treatments received 2/3,
 339 intermediate drought received 1/3 and extreme drought received no water. Pots were
 340 watered every other day with autoclaved water. Soil moisture was evaluated
 341 gravimetrically. Drought lasted 2 weeks followed by 2 weeks of recovery when pots
 342 were slowly rewetted to optimum moisture (65% WHC). Drought was repeated up to 3
 343 times depending on the drought frequency treatment. Control pots were always kept at
 344 65% WHC. Drought levels were compared for contextualisation to historical data of soil
 345 moisture (1991-2020) in England, made available by the CCI SM project^{59,60}. Minimum
 346 annual values of soil moisture were fitted to an extreme value distribution (Gumbel
 347 distribution) with the package “extRemes”⁶¹. The maximum drought experienced by
 348 treatments in each cycle was approximately 40% WHC for mild drought (~23%
 349 volumetric water content), which corresponded to the average values of soil moisture
 350 during the summer (Jun-Aug), 23% WHC for intermediate drought (~14% volumetric

351 water content), which resembled common summer drought events (once every 4 years),
 352 and 11% WHC for extreme drought (~7% volumetric water content), which simulated a
 353 once in a century drought. Extended Data Fig. 7a summarizes the evolution of WHC.
 354 WHC capacity was affected by the drought treatments (Extended Data Fig. 7b), so after
 355 each drought cycle, WHC was re-evaluated. The decrease of moisture retention capacity
 356 of soils after repeated drought has previously been documented^{23,62}.

357 Immediately after the last drought cycle, samples were collected to evaluate the
 358 resistance of the system. We consider the perturbation the entire drying/rewetting cycle,
 359 and thus, to evaluate the resistance we harvested the pots once the perturbation had
 360 ended (when all pots recovered the same WHC, 11 days after the start of the rewetting,
 361 Extended Data Fig. 7a). Additionally, pots were harvested over time, to evaluate the
 362 resilience of the system in the long term (1, 3 and 6 months after drought), which covers
 363 the length of the typical growing season for the study sites where soil was collected in
 364 the Yorkshire Dales (Met Office UK). 40 extra pots were harvested 1 month after
 365 drought to evaluate the adaptation of microbial growth characteristics to drought. Total
 366 number of pots: 200.

367 **Microbial community structure**

368 Soil samples were collected in Eppendorf tubes (approx. 0.25 g) and frozen at -80 °C
 369 just after sampling. DNA was extracted in frozen samples, without thawing, with
 370 PowerSoil DNA isolation Kit (Qiagen, Germany). DNA was sent to Macrogen
 371 sequencing service (Macrogen Inc., Korea), for Illumina sequencing (MiSeq v3). Fungal
 372 diversity was evaluated by ITS2 sequencing, using the primer pair 5.8S-Fun and ITS4-
 373 Fun⁶³. Bacterial diversity was evaluated by 16S V3-V4 sequencing, with primers
 374 Bakt_341F and Bakt_805R⁶⁴. Microbial community analysis was not done in samples

375 from the 3 months after drought time point. Alongside the samples, three extraction
376 blanks were included and a mock community sample for each primer pair: 19 strains
377 genomic DNA even mix from Bakker lab for fungi⁶⁵, and MSA-1000™ 10 strain even
378 mix genomic material for bacteria from the American Type Culture Collection (ATCC,
379 Manassas, US).

380 The quality of sequences was assessed using FASTQC⁶⁶. Primers were removed
381 using cutadapt⁶⁷ (fungi) or obitools⁶⁸ (bacteria). Low quality areas at the end of the
382 reads were trimmed using truncLen= c(275,200) (fungi) or truncLen= c(260,180) plus
383 10 additional nucleotides at the beginning of forward reads (bacteria). Sequences were
384 analysed using the DADA2 pipeline⁶⁹ with default parameters, except maxEE for fungi
385 which was set to 4 in each direction. After filtering and de-noising steps 77% (fungi) or
386 44% (bacteria) of the reads were retained, and 4,227 (fungi) or 16,924 (bacteria)
387 amplicon sequence variants (ASV) were identified. Taxonomic identification was
388 performed by IDTAXA taxonomic classification method in DECIPHER⁷⁰ package
389 using UNITE reference database (sh_general_release_dynamic_s_01.12.2017.fasta) for
390 fungi and SILVA database (SILVA_SSU_r132_March2018.RData) for bacteria.
391 Database was refined by four consecutive steps. 1) Renormalization to counteract the
392 problem of tag-switching⁷¹: for the abundant ASVs (≥ 100 reads), eliminate the reads of
393 the samples corresponding to a cumulative frequency of less than 3% for each particular
394 ASV. 2) Lulu algorithm to reduce the number of erroneous ASVs and achieve more
395 realistic biodiversity metrics⁷², setting the minimum ratio at 2 for fungi and minimum
396 match at 99% for bacteria, and the rest parameters set as default. The election of these
397 parameters was based on the results of the mock community sample. 3) Minimal
398 abundance filtering, by removing any reads that represent $< 0.02\%$ abundance in each
399 sample. 4) Blank correction, where ASVs were removed if the total abundance in blanks

divided by the total abundance in sample was greater than 10%, in each particular ASV. Finally, only fungi or bacteria sequences were retained. For fungal database, other eukaryotic reads that were removed represented the 0.01% of the reads. For bacteria, taxa unclassified at domain level represented the 2.69% of the reads, Archaea reads represented the 0.004% and chloroplast sequences represented the 0.0007%. All of them were removed. Final databases contained 2,760 ASVs and 4,316,693 reads for fungi, and 7,198 ASVs and 2,392,480 reads for bacteria. Sampling depth varied from 25,873–53,007 (mean 36,275) reads in fungi and 12,595–58,150 (mean 19,937) reads in bacteria, and it was equally distributed among drought treatments. Rarefaction curves were plotted using the *rarecurve* function in “vegan”⁷³, and these indicated that sampling depth was sufficient for all samples, plateauing at around 7,500 reads.

Soil enzymes

β -glucosidase (GLC), cellobiohydrolase (CBH), xylosidase (XYL), *N*-acetylglucosaminidase (NAG) and acid phosphatase (PHO) were measured photometrically according to Jackson *et al.* 2013⁷⁴, with modifications. 3.75 g of sieved soil was suspended in 5 mL of sodium acetate buffer (50 mM, pH 5.0). 150 μ L of soil slurry were introduced into a 96-well deepwell block and mixed with 150 μ L of a saturating substrate solution: 25 mM *p*NP- β -glucopyranoside for GLC, 2 mM *p*NP- β -D-cellobioside for CBH, 25 mM *p*NP- β -xylopyranoside for XYL, 5 mM *p*NP-N-acetyl- β -D-glucosaminide for NAG and 5 mM *p*NP-phosphate disodium salt hexahydrate for PHO. Plates were incubated at 18 °C for 0.5 h (PHO), 1.5 h (GLC), 3.5 h (XYL and NAG) or 4 h (CBH) under continuous shaking. Blocks were centrifuged (2900 g, 5 min), 100 μ L of the supernatant pipetted into transparent 96-well plates and mixed with 200 μ L of 50 mM NaOH solution. Absorbance was measured at 405 nm in a plate reader

(EZ400 Research, Biochrom, Germany) and corrected for soil and substrate colouration.
Reported activity is the mean of four analytical replicates.

Urease (URE) was measured by the optimised high throughput method⁷⁵ without modifications. Absorbance was evaluated in a microplate reader (EZ400 Research, Biochrom, Germany) and reported activity is the mean of four analytical replicates. Phenoloxidase (POX) and peroxidase (PER) activities were measured photometrically following Sinsabaugh & Linkins 1988⁷⁶ method, with modifications. 0.25 g of soil were suspended in 25 ml of sodium acetate buffer (50 mM, pH 5.0). 0.4 ml of soil slurry were extracted under continuous shaking and mixed (1:1) with a 20 mM L-3,4-dihydroxyphenylalanine (L-DOPA) solution in a deep-well block. Blocks were shaken for 10 min and centrifuged (2900 g, 5 min). 250 µl of the supernatant were pipetted into transparent 96-well plates. For peroxidase activity, wells additionally received 10 µl of a 0.3% H₂O₂ solution. Absorbance was measured at 450 nm in a plate reader (EZ400 Research, Biochrom, Germany) at the starting time point (t₀), after 1.5 h (t₁ for PER) and after 20 h (t₁ for POX) at 18 °C. Enzyme activity was calculated from the difference in absorption between the two time-points divided by L-DOPA molar extinction coefficient (7.9 µmol⁻¹ 77). For PER activity, POX activity was subtracted. Reported activity is the mean of three analytical replicates.

Nutrient pools

Different nutrient pools were measured by means of soil extractions with different extracting solutions depending on the nutrient and the pool of interest⁷⁸. All soil nutrients were evaluated in duplicates, and reported values are the mean of those two analytical replicates. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were evaluated in water extracts (5 g soil in 28 ml MilliQ water) and plant

448 available nitrogen (ammonium and nitrate) were evaluated in KCl extracts (2.5 g soil in
449 12.5 ml 1M KCl). In both cases, soil with extracting solutions were horizontally shaken
450 at 200 rpm for 30 minutes and filtered through Whatman n° 42 filter papers (KCl
451 extracts) or 0.45 µm syringe filters (H₂O extracts). N pools were measured in AA3 HR
452 Auto Analyser (Seal Analytical, UK) while C pools were measured in 5000A TOC-L
453 analyser (Shimadzu, Japan). Plant available P was extracted with acetic acid solution (1
454 g soil + 25 ml 2.5% acetic acid) and detected by molybdate colorimetry using AA3 HR
455 Auto Analyzer (Seal Analytical, UK).

456 Organic P was estimated by evaluation of available phosphate before and after
457 sample ignition⁷⁹. Two portions of dry soil (2 g) were weighed. One of them was burned
458 at 550°C for 4 h in a furnace. Afterwards, both samples were extracted with 50 ml 0.5 M
459 H₂SO₄ by horizontally shaking at 120 rpm for 16 h. Extracts were filtered (Whatman
460 No. 42) and phosphate was evaluated by the ascorbic acid method⁷⁹. After sample
461 neutralization with NaOH, the colour reaction was carried out and the amount of blue
462 was evaluated by absorbance at 880 nm in a CLARIOstar plate reader (BMG Labtech,
463 Germany).

464 Microbial biomass C and N was measured using the fumigation–extraction
465 techniques^{80,81}. 2.5 g of soil were fumigated with CHCl₃ for 24 h. Soluble C and N were
466 extracted from the fumigated and from un-fumigated samples with 12.5 ml 0.5 M
467 K₂SO₄. Soil + extracting solution were shaken and filtered (Whatman No. 42) and total
468 C and N were analysed in TOC and AA, respectively. Microbial C and N flush
469 (difference between fumigated and un-fumigated samples) were converted to microbial
470 biomass using k_{EC} factor of 0.35⁸² and k_{EN} factor of 0.54⁸⁰.

471 Microbial P was estimated with the hexanol fumigation and extraction with anion-
472 exchange membranes method⁸³. Membrane strips (1 x 4 cm) were prepared by shaking

473 in 0.5 M NaHCO₃. For each sample, three portions of fresh soil (2 g) were weighed into
 474 50 ml tubes: unfumigated, fumigated and spiked samples. In each tube, 30 ml deionized
 475 water and three anion-exchange membrane strips were added. Fumigated samples
 476 received 1 ml of hexanol while spiked samples received 1 ml of a 20 mg ml⁻¹ P solution.
 477 All tubes were shaken for 24 h. The membranes were then removed and rinsed with
 478 deionized water and phosphate recovered by shaking for 1 h in 20 ml of 0.25 M H₂SO₄,
 479 with detection at 880 nm by automated molybdate colorimetry using AA3 HR Auto
 480 Analyzer (Seal Analytical, UK). Microbial phosphorus were calculated as the difference
 481 between the fumigated and un-fumigated samples, corrected by the sorption percentage
 482 (spiked samples) and transformed using a k_{EP} factor of 0.40⁸⁴.

483 **Adaptation of microbial growth characteristics to drought**

484 Adaptation of soil bacterial and fungal community characteristics to drought was
 485 evaluated by assessing their growth and respiration rate responses to a subsequent
 486 drying/rewetting cycle in soil samples that had been recovering for one month after
 487 drought. We did two complementary tests: first, we assessed the response to a full
 488 drying/rewetting cycle, to compare the ability of microbial communities to recover after
 489 rewetting and secondly, we evaluated moisture dependences to test the ability of
 490 microbes to withstand a lack of moisture. For the first test, soil samples were
 491 completely air dried for two days and then rewetted to 60% WHC. After rewetting,
 492 microbial growth rates were measured with a high temporal resolution of approximately
 493 6 h for a 160 h period (more frequent at the beginning and every 24 h afterwards, 12
 494 time points in total). Bacterial and fungal growth rates were assessed via radioisotope
 495 incorporation⁸⁵. Bacterial growth rates were evaluated by ³H-leucine incorporation into
 496 proteins⁸⁶ while fungal growth rates were measured by ¹⁴C-acetate incorporation into
 497 ergosterol⁸⁷. To measure soil respiration, 1.0 g of soil was weighed into 20-ml glass

vials, which were purged with pressurised air, sealed with crimp caps and incubated for 6 h in the dark at 17°C. CO₂ production was measured using a gas chromatograph equipped with a methaniser and an FID detector. To evaluate the moisture dependence of microbial processes, microbial growth and respiration rates were measured during the drying down of the soils. Then, IC₁₀ (i.e. the moisture level at which growth and respiration rates were reduced by 10%) was determined, which allowed comparison of the drought tolerance of the different microbial processes²².

Statistical analyses

All statistical analyses were done in R v4.0⁸⁸. To evaluate microbial community structure, we investigated alpha diversity, community structure with ordination analyses, proportion of different taxa and functional guilds (only for fungi) and we performed indicator species analysis. Due to the issues associated with using rarefied or relative abundance data for diversity tests and differential abundance analyses⁸⁹, different approaches or data transformations were applied for each analysis.

Alpha diversity was calculated estimating the unobserved diversity⁹⁰. Richness was estimated with “breakaway” package⁹¹ and Shannon index with “DivNet” package⁹². Community structure was explored with a variance stabilization transformation (VST) of data, using “DeSeq2” package⁹³. PERMANOVA analyses using Euclidean distance of transformed data were performed to evaluate differences among drought treatments, with soil as random factor, using *adonis* function in “vegan” package⁷³. To better visualise the effects of the drought treatments, a correction to eliminate the soil effect was performed with *removeBatchEffect* function in “limma” package⁹⁴. A PCA analysis was then performed in the corrected database using “vegan”⁷³. To predict functional guilds and trophic modes from the fungal taxonomic data we used FUNGuild⁹⁵. Only

those assignments considered highly probable or probable were used, while possible assignments were discarded. Assignments with multiple and contradictory guilds to the same taxa were also discarded. In average, 29.1% of the taxa (774 ASVs) were assigned and used in this analysis. Of those, 82 were assigned to order level, 158 to family level and 534 to genus level. For relative abundance graphs, only taxa (phylum, order or family) with relative abundances >1% across all samples were plotted.

Indicator species analysis was performed using *multipatt* function in the “indicspecies” package⁹⁶ to identify bacterial and fungal taxa associated with drought and control treatments. We tested for indicator taxa depending on the intensity of drought, comparing the control treatment with the extreme drought and some specific combinations (control+mild, control+mild+intermediate, and intermediate+extreme drought). Prior to analysis, ASVs were agglomerated at genus level using *tax_glom* in “phyloseq” package⁹⁷, and libraries were normalised using cumulative sum scaling (CSS)⁹⁸, using *phyloseq_transform_css* function in “metagMisc” package⁹⁹. CSS-normalised abundance heatmaps were produced using *plot_heatmap* function in “phyloseq” package⁹⁷.

Resistance and resilience of soil functions were evaluated with a linear regression analysis between the value of the variable under drought and time after drought (in weeks). We used relative data in percentage (i.e., value drought pot/value control pot × 100), and we performed individual analyses for each of the nine drought intensity and drought frequency combinations. The value of the intercept was used as a resistance index (RS), as it represents the relative difference of the drought treatment with the control just after drought. RS can be directly interpreted as the % change compared to the control treatment. Additionally, we used the value of the slope as a resilience index (RL). This value was adjusted depending on the direction of change after drought with

the formula: if intercept < 100, then RL = slope; if intercept > 100, then RL = -slope. Thus, a positive RL indicates a recovery of the system; variables in drought pots are getting more similar to those in control pots. On the other hand, RL = 0 reflects no change (no resilience), with drought pots showing the same difference with the control ones that what it was at the end of the drought. Moreover, a negative RL can be interpreted as delayed response to drought or a continuous negative effect even when the perturbation has finished, as negative RL mean a bigger difference with the control over time than what it was at the end of the drought. RL values can be directly interpreted as percentage recovery/change per week.

Soil functional data (soil extracellular enzymes and nutrient pools) were used in a non-metric multidimensional scaling (NMDS) ordination analysis, performed with the function *metaMDS* in “vegan”⁷³, using relative data (value/average of the control at each sampling date). Multifunctionality index¹⁰⁰ was calculated with all the soil enzymatic activities, which represent the organic matter decomposition capacity of soils. Data were standardised by z transformation separately for each sampling time, which removed overall differences between harvest time points¹⁰¹. Subsequently, the average of all standardised values was used as the multifunctionality index.

The effects of drought intensity and frequency in all variables were analysed by linear mixed effect models (LME) with drought intensity and frequency as fixed factors and soil as random factor, with *lme* function in “nlme” package¹⁰². To obtain a balanced design, control pots were removed from the analysis. Normality of the residuals and homoscedasticity were confirmed with Anderson-Darling test and Levene test, respectively. If needed, natural logarithmic or square root transformations were applied. If the assumptions were still not met, Kruskal-Wallis test was performed instead. *P*

571 values after multiple comparisons (for bacterial and fungal taxa) were adjusted with the
572 Benjamini-Hochberg adjustment^{103,104} using *p.adjust* function.

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574 REFERENCES

- 575 1. Jansson, J. K. & Hofmockel, K. S. Soil microbiomes and climate change. *Nat.*
576 *Rev. Microbiol.* **18**, 35–46 (2020).
- 577 2. IPCC. *Climate Change 2021: The Physical Science Basis. Working Group I*
578 *contribution to the Sixth Assessment Report of the Intergovernmental Panel on*
579 *Climate Change.* (2021).
- 580 3. Naylor, D. & Coleman-Derr, D. Drought stress and root-associated bacterial
581 communities. *Front. Plant Sci.* **8**, 1–16 (2018).
- 582 4. Schimel, J. P. Life in dry soils: effects of drought on soil microbial communities
583 and processes. *Annu. Rev. Ecol. Evol. Syst.* **49**, 409–432 (2018).
- 584 5. Evans, S. E. & Wallenstein, M. D. Climate change alters ecological strategies of
585 soil bacteria. *Ecol. Lett.* **17**, 155–164 (2014).
- 586 6. Kaisermann, A., de Vries, F. T., Griffiths, R. I. & Bardgett, R. D. Legacy effects
587 of drought on plant–soil feedbacks and plant–plant interactions. *New Phytol.* **215**,
588 1413–1424 (2017).
- 589 7. Bardgett, R. D. & Caruso, T. Soil microbial community responses to climate
590 extremes: resistance, resilience and transitions to alternative states. *Philos. Trans.*
591 *R. Soc. B Biol. Sci.* **375**, 20190112 (2020).
- 592 8. Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. & Walker, B. Catastrophic
593 shifts in ecosystems. *Nature* **413**, 591–596 (2001).
- 594 9. Wernberg, T. *et al.* Climate-driven shift of a temperate marine ecosystem.
595 *Science (80-.).* **353**, 169–172 (2016).

- 596 10. Yodzis, P. The stability of real ecosystems. *Nature* **289**, 674–676 (1981).
- 597 11. Bush, T. *et al.* Oxic-anoxic regime shifts mediated by feedbacks between
598 biogeochemical processes and microbial community dynamics. *Nat. Commun.* **8**,
599 789 (2017).
- 600 12. Fletcher, M.-S., Wood, S. W. & Haberle, S. G. A fire driven shift from forest to
601 non-forest: evidence for alternative stable states? *Ecology* **95**, 2504–2513 (2014).
- 602 13. Abella, S. R., Gentilcore, D. M. & Chiquoine, L. P. Resilience and alternative
603 stable states after desert wildfires. *Ecol. Monogr.* **91**, e01432 (2021).
- 604 14. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R.
605 Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220–
606 230 (2012).
- 607 15. Hartman, A. L. *et al.* Human gut microbiome adopts an alternative state
608 following small bowel transplantation. *Proc. Natl. Acad. Sci. U. S. A.* **106**,
609 17187–17192 (2009).
- 610 16. Bardgett, R. D. & Van Der Putten, W. H. Belowground biodiversity and
611 ecosystem functioning. *Nature* **515**, 505–511 (2014).
- 612 17. Bender, E. A. ., Case, T. J. . & Gilpin, M. E. . Perturbation experiments in
613 community ecology: theory and practice. *Ecology* **65**, 1–13 (1984).
- 614 18. Barnard, R. L., Osborne, C. A. & Firestone, M. K. Responses of soil bacterial
615 and fungal communities to extreme desiccation and rewetting. *ISME J.* **7**, 2229–
616 2241 (2013).
- 617 19. Leizeaga, A., Hicks, L. C., Manoharan, L., Hawkes, C. V. & Rousk, J. Drought

- 618 legacy affects microbial community trait distributions related to moisture along a
619 savannah grassland precipitation gradient. *J. Ecol.* 1–16 (2020)
620 doi:10.1111/1365-2745.13550.
- 621 20. de Vries, F. T. *et al.* Soil bacterial networks are less stable under drought than
622 fungal networks. *Nat. Commun.* **9**, 3033 (2018).
- 623 21. Bouskill, N. J. *et al.* Pre-exposure to drought increases the resistance of tropical
624 forest soil bacterial communities to extended drought. *ISME J.* **7**, 384–394
625 (2013).
- 626 22. de Nijs, E. A., Hicks, L. C., Leizeaga, A., Tietema, A. & Rousk, J. Soil microbial
627 moisture dependences and responses to drying–rewetting: the legacy of 18 years
628 drought. *Glob. Chang. Biol.* **25**, 1005–1015 (2019).
- 629 23. Robinson, D. A. *et al.* Experimental evidence for drought induced alternative
630 stable states of soil moisture. *Sci. Rep.* **6**, 20018 (2016).
- 631 24. Jassey, V. E. J. *et al.* Tipping point in plant-fungal interactions under severe
632 drought causes abrupt rise in peatland ecosystem respiration. *Glob. Chang. Biol.*
633 **24**, 972–986 (2018).
- 634 25. Todman, L. C. *et al.* Evidence for functional state transitions in intensively-
635 managed soil ecosystems. *Sci. Rep.* **8**, 11522 (2018).
- 636 26. Petraitis, P. S. & Dudgeon, S. R. Detection of alternative stable states in marine
637 communities. *J. Exp. Mar. Bio. Ecol.* **300**, 343–371 (2004).
- 638 27. Preece, C., Verbruggen, E., Liu, L., Weedon, J. T. & Peñuelas, J. Effects of past
639 and current drought on the composition and diversity of soil microbial

- communities. *Soil Biol. Biochem.* **131**, 28–39 (2019).
28. Lennon, J. T., Aanderud, Z. T., Lehmkuhl, B. K. & Schoolmaster, D. R. Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* **93**, 1867–1879 (2012).
29. Pérez Castro, S., Cleland, E. E., Wagner, R., Sawad, R. Al & Lipson, D. A. Soil microbial responses to drought and exotic plants shift carbon metabolism. *ISME J.* **13**, 1776–1787 (2019).
30. Curiel Yuste, J. *et al.* Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. *Soil Biol. Biochem.* **69**, 223–233 (2014).
31. Jang, S. W. *et al.* Re-Analysis of 16S amplicon sequencing data reveals soil microbial population shifts in rice fields under drought condition. *Rice* **13**, 44 (2020).
32. Brewer, T. E., Handley, K. M., Carini, P., Gilbert, J. A. & Fierer, N. Genome reduction in an abundant and ubiquitous soil bacterium ‘*Candidatus* Udaeobacter copiosus’. *Nat. Microbiol.* **2**, 16198 (2016).
33. Thijs, S. *et al.* Potential for plant growth promotion by a consortium of stress-tolerant 2,4-dinitrotoluene-degrading bacteria: isolation and characterization of a military soil. *Microb. Biotechnol.* **7**, 294–306 (2014).
34. Ofek, M., Hadar, Y. & Minz, D. Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PLoS One* **7**, e40117 (2012).
35. Viitamäki, S. The activity and functions of soil microbial communities across a

- 662 climate gradient in Finnish subarctic. (University of Helsinki, 2019).
- 663 36. Moreno, J. L., Torres, I. F., García, C., López-Mondéjar, R. & Bastida, F. Land
664 use shapes the resistance of the soil microbial community and the C cycling
665 response to drought in a semi-arid area. *Sci. Total Environ.* **648**, 1018–1030
666 (2019).
- 667 37. Bapiri, A., Bååth, E. & Rousk, J. Drying-rewetting cycles affect fungal and
668 bacterial growth differently in an arable soil. *Microb. Ecol.* **60**, 419–428 (2010).
- 669 38. Fierer, N. & Jackson, R. B. The diversity and biogeography of soil bacterial
670 communities. *Proc. Natl. Acad. Sci.* **103**, 626–631 (2006).
- 671 39. Rousk, J. *et al.* Soil bacterial and fungal communities across a pH gradient in an
672 arable soil. *ISME J.* **4**, 1340–1351 (2010).
- 673 40. Tedersoo, L. *et al.* Global diversity and geography of soil fungi. *Science* (80-.).
674 **346**, 1256688 (2014).
- 675 41. Xiao, W., Chen, X., Jing, X. & Zhu, B. A meta-analysis of soil extracellular
676 enzyme activities in response to global change. *Soil Biol. Biochem.* **123**, 21–32
677 (2018).
- 678 42. Burns, R. G. *et al.* Soil enzymes in a changing environment: Current knowledge
679 and future directions. *Soil Biol. Biochem.* **58**, 216–234 (2013).
- 680 43. Ochoa-Hueso, R. *et al.* Drought consistently alters the composition of soil fungal
681 and bacterial communities in grasslands from two continents. *Glob. Chang. Biol.*
682 (2018) doi:10.1111/gcb.14113.
- 683 44. Acosta-Martinez, V., Moore-Kucera, J., Cotton, J., Gardner, T. & Wester, D. Soil

- 684 enzyme activities during the 2011 Texas record drought/heat wave and
685 implications to biogeochemical cycling and organic matter dynamics. *Appl. Soil*
686 *Ecol.* **75**, 43–51 (2014).
- 687 45. Williams, M. A. & Xia, K. Characterization of the water soluble soil organic pool
688 following the rewetting of dry soil in a drought-prone tallgrass prairie. *Soil Biol.*
689 *Biochem.* **41**, 21–28 (2009).
- 690 46. Austin, A. T. *et al.* Water pulses and biogeochemical cycles in arid and semiarid
691 ecosystems. *Oecologia* **141**, 221–235 (2004).
- 692 47. Fierer, N. & Schimel, J. P. Effects of drying-rewetting frequency on soil carbon
693 and nitrogen transformations. *Soil Biol. Biochem.* **34**, 777–787 (2002).
- 694 48. Hueso, S., García, C. & Hernández, T. Severe drought conditions modify the
695 microbial community structure, size and activity in amended and unamended
696 soils. *Soil Biol. Biochem.* **50**, 167–173 (2012).
- 697 49. Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R. & Richter, A. Experimental
698 drought reduces the transfer of recently fixed plant carbon to soil microbes and
699 alters the bacterial community composition in a mountain meadow. *New Phytol.*
700 **201**, 916–927 (2014).
- 701 50. Dijkstra, F. A., He, M., Johansen, M. P., Harrison, J. J. & Keitel, C. Plant and
702 microbial uptake of nitrogen and phosphorus affected by drought using ¹⁵N and
703 ³²P tracers. *Soil Biol. Biochem.* **82**, 135–142 (2015).
- 704 51. Rousk, J., Smith, A. R. & Jones, D. L. Investigating the long-term legacy of
705 drought and warming on the soil microbial community across five European
706 shrubland ecosystems. *Glob. Chang. Biol.* **19**, 3872–3884 (2013).

- 707 52. Hicks, L. C., Ang, R., Leizeaga, A. & Rousk, J. Bacteria constrain the fungal
708 growth response to drying-rewetting. *Soil Biol. Biochem.* **134**, 108–112 (2019).
- 709 53. de Vries, F. T. & Shade, A. Controls on soil microbial community stability under
710 climate change. *Front. Microbiol.* **4**, 265 (2013).
- 711 54. Fukami, T. Historical contingency in community assembly: integrating niches,
712 species pools, and priority effects. *Annu. Rev. Ecol. Evol. Syst.* **46**, 1–23 (2015).
- 713 55. Kreyling, J. *et al.* To replicate, or not to replicate – that is the question: how to
714 tackle nonlinear responses in ecological experiments. *Ecol. Lett.* **21**, 1629–1638
715 (2018).
- 716 56. Meisner, A., Leizeaga, A., Rousk, J. & Bååth, E. Partial drying accelerates
717 bacterial growth recovery to rewetting. *Soil Biol. Biochem.* **112**, 269–276 (2017).
- 718 57. Shade, A. *et al.* Fundamentals of microbial community resistance and resilience.
719 *Front. Microbiol.* **3**, 417 (2012).
- 720 58. Williams, A. & de Vries, F. T. Plant root exudation under drought: implications
721 for ecosystem functioning. *New Phytol.* **225**, 1899–1905 (2020).
- 722 59. Dorigo, W. *et al.* ESA CCI Soil Moisture for improved Earth system
723 understanding: State-of-the art and future directions. *Remote Sens. Environ.* **203**,
724 185–215 (2017).
- 725 60. Gruber, A., Scanlon, T., Van Der Schalie, R., Wagner, W. & Dorigo, W.
726 Evolution of the ESA CCI Soil Moisture climate data records and their
727 underlying merging methodology. *Earth Syst. Sci. Data* **11**, 717–739 (2019).
- 728 61. Gilleland, E. & Katz, R. W. extRemes 2.0: An Extreme Value Analysis Package

- 729   in R. *J. Stat. Softw.* **72**, 1–39 (2016).
- 730   62.   Ahmadi, K. *et al.* Effects of rhizosphere wettability on microbial biomass,
731   enzyme activities and localization. *Rhizosphere* **7**, 35–42 (2018).
- 732   63.   Taylor, D. *et al.* Accurate estimation of fungal diversity and abundance through
733   improved lineage-specific primers optimized for Illumina amplicon sequencing.
734   *Appl. Environ. Microbiol.* **82**, 7217–7226 (2016).
- 735   64.   Herlemann, D. P. R. *et al.* Transitions in bacterial communities along the 2000
736   km salinity gradient of the Baltic Sea. *ISME J.* **5**, 1571–1579 (2011).
- 737   65.   Bakker, M. G. A fungal mock community control for amplicon sequencing
738   experiments. *Mol. Ecol. Resour.* **18**, 541–556 (2018).
- 739   66.   Andrews, S. FastQC: A quality control tool for high throughput sequence data.
740   <http://www.bioinformatics.babraham.ac.uk/projects/>. (2010).
- 741   67.   Martin, M. Cutadapt removes adapter sequences from high-throughput
742   sequencing reads. *EMBnet.journal* **17**, 10–12 (2011).
- 743   68.   Boyer, F. *et al.* OBITOOLS: A UNIX-inspired software package for DNA
744   metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182 (2016).
- 745   69.   Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina
746   amplicon data. *Nat. Methods* **13**, 581–583 (2016).
- 747   70.   Wright, E. S. Using DECIPHER v2.0 to analyze big biological sequence data in
748   R. *R J.* **8**, 352–359 (2016).
- 749   71.   Wangenstein, O. S., Palacín, C., Guardiola, M. & Turon, X. DNA metabarcoding
750   of littoral hard-bottom communities: high diversity and database gaps revealed

- 751   by two molecular markers. *PeerJ* **6**, e4705 (2018).
- 752   72.   Frøslev, T. G. *et al.* Algorithm for post-clustering curation of DNA amplicon
753   data yields reliable biodiversity estimates. *Nat. Commun.* **8**, 1188 (2017).
- 754   73.   Oksanen, J. *et al.* vegan: community ecology package. R package version 2.5.
755   (2020).
- 756   74.   Jackson, C. R., Tyler, H. L. & Millar, J. J. Determination of microbial
757   extracellular enzyme activity in waters, soils, and sediments using high
758   throughput microplate assays. *J. Vis. Exp.* **80**, 1–9 (2013).
- 759   75.   Cordero, I., Snell, H. & Bardgett, R. D. High throughput method for measuring
760   urease activity in soil. *Soil Biol. Biochem.* **134**, 72–77 (2019).
- 761   76.   Sinsabaugh, R. L. & Linkins, A. E. Exoenzyme activity associated with lotic
762   epilithon. *Freshw. Biol.* **20**, 249–261 (1988).
- 763   77.   DeForest, J. L. The influence of time, storage temperature, and substrate age on
764   potential soil enzyme activity in acidic forest soils using MUB-linked substrates
765   and l-DOPA. *Soil Biol. Biochem.* **41**, 1180–1186 (2009).
- 766   78.   Rhymes, J. *et al.* Are researchers following best storage practices for measuring
767   soil biochemical properties? *SOIL* **7**, 95–106 (2021).
- 768   79.   Kuo, S. Phosphorus. in *Methods of soil analysis, Part 3. Chemical methods* (eds.
769   Sparks, D. L., Page, A. L., Helmke, P. A. & Loeppert, R. H.) 869–919 (Soil
770   Science Society of America, American Society of Agronomy, 1996).
- 771   80.   Brookes, P. C., Landman, A., Pruden, G. & Jenkinson, D. S. Chloroform
772   fumigation and the release of soil nitrogen: A rapid direct extraction method to

- 773   measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **17**, 837–842
- 774   (1985).
- 775   81.   Vance, E. D., Brookes, P. C. & Jenkinson, D. S. An extraction method for
- 776   measuring soil microbial biomass C. *Soil Biol. Biochem.* **19**, 703–707 (1987).
- 777   82.   Sparling, G. P., Feltham, C. W., Reynolds, J., West, A. W. & Singleton, P.
- 778   Estimation of soil microbial C by a fumigation extraction method: use on soils of
- 779   high organic matter content, and a reassessment of the k_{EC} -factor. *Soil Biol.*
- 780   *Biochem.* **22**, 301–307 (1990).
- 781   83.   Turner, B. L. & Romero, T. E. Stability of hydrolytic enzyme activity and
- 782   microbial phosphorus during storage of tropical rain forest soils. *Soil Biol.*
- 783   *Biochem.* **42**, 459–465 (2010).
- 784   84.   Brookes, P. C., Powlson, D. S. & Jenkinson, D. S. Measurement of microbial
- 785   biomass phosphorus in soil. *Soil Biol. Biochem.* **14**, 319–329 (1982).
- 786   85.   Rousk, J. & Bååth, E. Growth of saprotrophic fungi and bacteria in soil. *FEMS*
- 787   *Microbiol. Ecol.* **78**, 17–30 (2011).
- 788   86.   Bååth, E. Measurement of protein synthesis by soil bacterial assemblages with
- 789   the leucine incorporation technique. *Biol. Fertil. Soils* **17**, 147–153 (1994).
- 790   87.   Bååth, E. Estimation of fungal growth rates in soil using ^{14}C -acetate
- 791   incorporation into ergosterol. *Soil Biol. Biochem.* **33**, 2011–2018 (2001).
- 792   88.   R Core Team, _ . *R: a language and environment for statistical computing*. (R
- 793   Foundation for Statistical Computing, 2020).
- 794   89.   McMurdie, P. J. & Holmes, S. Waste not, want not: why rarefying microbiome

- 795 data is inadmissible. *PLoS Comput. Biol.* **10**, e1003531 (2014).
- 796 90. Willis, A. D. Rarefaction, alpha diversity, and statistics. *Front. Microbiol.* **10**,
797 2407 (2019).
- 798 91. Willis, A. *et al.* breakaway: Species Richness Estimation and Modeling. R
799 package version 4.7.3. (2020).
- 800 92. Willis, A. D. & Martin, B. D. Estimating diversity in networked ecological
801 communities. *Biostatistics* (2020).
- 802 93. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and
803 dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 1–21 (2014).
- 804 94. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-
805 sequencing and microarray studies. *Nucleic Acids Res.* **43**, e47 (2015).
- 806 95. Nguyen, N. H. *et al.* FUNGuild: An open annotation tool for parsing fungal
807 community datasets by ecological guild. *Fungal Ecol.* **20**, 241–248 (2016).
- 808 96. De Cáceres, M. & Legendre, P. Associations between species and groups of sites:
809 indices and statistical inference. *Ecology* **90**, 3566–3574 (2009).
- 810 97. Mcmurdie, P. J. & Holmes, S. phyloseq: an R package for reproducible
811 interactive analysis and graphics of microbiome census data. *PLoS One* **8**,
812 e61217 (2013).
- 813 98. Paulson, J. N., Colin Stine, O., Corrada Bravo, H. & Pop, M. Differential
814 abundance analysis for microbial marker-gene surveys. *Nat. Methods* **10**, 1200–
815 1202 (2013).
- 816 99. Mikryukov, V. metagMisc: Miscellaneous functions for metagenomic analysis.

- 817 (2020).
- 818 100. Maestre, F. T. *et al.* Plant species richness and ecosystem multifunctionality in
819 global drylands. *Science* (80-.). **335**, 214–218 (2012).
- 820 101. Wagg, C., Bender, S. F., Widmer, F. & Van Der Heijden, M. G. A. Soil
821 biodiversity and soil community composition determine ecosystem
822 multifunctionality. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 5266–5270 (2014).
- 823 102. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Team, R. C. nlme: Linear and
824 Nonlinear Mixed Effects Models. R package version 3.1-149. (2020).
- 825 103. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical
826 and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289–300
827 (1995).
- 828 104. Jafari, M. & Ansari-Pour, N. Why, when and how to adjust your P values? *Cell J.*
829 **20**, 604–607 (2019).

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842 **AUTHOR CONTRIBUTIONS**

843 IC and RDB conceived the study and designed the experiment, with inputs from JR.
844 IC performed nutrient analyses, enzyme assays, and microbial sequencing work. AL
845 and LH performed microbial growth rates measurements. IC analysed the data and
846 wrote the manuscript, with significant contributions from all authors.

847 **COMPETING INTERESTS**

848 We declare no competing interests.

849 FIGURE CAPTIONS

850 **Fig. 1 | Effects of drought on soil microbial community structure. a-d**, Alpha
851 diversity 6 months after drought (mean $\pm$ error of estimated values). **e-f**, Community
852 structure. The first two axes explained 17.3% (bacteria) and 5.4% (fungi) of the
853 variance. **g**, Percentage variance explained in PERMANOVA analyses including all the
854 treatments (global) or just one plus the control. **i-j**, Relative abundance of microbial
855 families. Data=mean (n=4). sg = subgroup. Significance of drought intensity (I),
856 frequency (F) and time point (T) is shown (**a-g**): *p<0.05, **p<0.01, ***p<0.001, or
857 significance of drought treatment for each sampling time (**i-j**): $\bar{p}$ >0.05, *p<0.05.

858 **Fig. 2 | Effects of drought on soil function. a,b**, Resistance and resilience indexes.
859 Asterisks indicate significant change (value $\neq$ 0, p<0.05). **c,d**, Functional structure of
860 soils. Mean $\pm$ error (n=4). Significance of PERMANOVA analysis evaluating the
861 effects of drought intensity (I) and frequency (F) is shown: *p<0.05, **p<0.01,
862 ***p<0.001. Differences in data dispersion (dd) among groups is also shown (^{ns}: non-
863 significant). GLC: β -glucosidase, CBH: cellobiohydrolase, XYL: xylosidase, NAG: N-
864 acetylglucosaminidase, PHO: acid phosphatase, POX: phenoloxidase, PER: peroxidase,
865 URE: urease, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, TOP:
866 total organic phosphorus, C_{mic}: microbial carbon, N_{mic}: microbial nitrogen, P_{mic}:
867 microbial phosphorus.

868 **Fig. 3 | Adaptation of microbial growth characteristics to drought.** Growth
869 responses to a further drying/rewetting cycle of soils with different drought history, one
870 month after drought. Significance of LME evaluating the effects of drought intensity (I)
871 and frequency (F) is shown: *p<0.05, **p<0.01, ***p<0.001.

872

Fig. 1 | Effects of drought on soil microbial community structure. a-d, Alpha diversity 6 months after drought (mean $\pm$ error of estimated values). **e-f**, Community structure. The first two axes explained 17.3% (bacteria) and 5.4% (fungi) of the variance. **g**, Percentage variance explained in PERMANOVA analyses including all the treatments (global) or just one plus the control. **i-j**, Relative abundance of microbial families. Data=mean ($n=4$). sg = subgroup. Significance of drought intensity (I), frequency (F) and time point (T) is shown (**a-g**): * $p<0.05$, ** $p<0.01$, *** $p<0.001$, or significance of drought treatment for each sampling time (**i-j**): - $p>0.05$, * $p<0.05$.

Fig. 2 | Effects of drought on soil function. a,b, Resistance and resilience indexes. Asterisks indicate significant change (value $\neq 0$, $p < 0.05$). **c,d,** Functional structure of soils. Mean $\pm$ error ($n=4$). Significance of PERMANOVA analysis evaluating the effects of drought intensity (I) and frequency (F) is shown: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Differences in data dispersion (dd) among groups is also shown (^{ns}: non-significant). GLC: β -glucosidase, CBH: cellobiohydrolase, XYL: xylosidase, NAG: *N*-acetylglucosaminidase, PHO: acid phosphatase, POX: phenoloxidase, PER: peroxidase, URE: urease, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, TOP: total organic phosphorus, C_{mic}: microbial carbon, N_{mic}: microbial nitrogen, P_{mic}: microbial phosphorus.

Fig. 3 | Adaptation to drought of microbial community traits.

Growth responses to a further drying/rewetting cycle of soils with different drought history, one month after drought. Significance of LME evaluating the effects of drought intensity (I) and frequency (F) is shown: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.