

Land-use drives the temporal stability and magnitude of soil microbial functions and modulates climate effects

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Abstract. Soil microbial community functions are essential indicators of ecosystem multifunctionality in managed land-use systems. Going forward, the development of adaptation strategies and predictive models under future climate scenarios will require a better understanding of how both land-use and climate disturbances influence soil microbial functions over time. Between March and November 2018, we assessed the effects of climate change on the magnitude and temporal stability of soil basal respiration, soil microbial biomass and soil functional diversity across a range of land-use types and intensities in a large-scale field experiment. Soils were sampled from five common land-use types including conventional and organic croplands, intensive and extensive meadows, and extensive pastures, under ambient and projected future climate conditions (reduced summer precipitation and increased temperature) at the Global Change Experimental Facility (GCEF) in Bad Lauchstädt, Germany. Land-use and climate treatment interaction effects were significant in September, a month when precipitation levels slightly rebounded following a period of drought in central Germany: compared to ambient climate, in future climate treatments, basal respiration declined in pastures and increased in intensive meadows, functional diversity declined in pastures and croplands, and respiration-to-biomass ratio increased in intensive and extensive meadows. Low rainfall between May and August likely strengthened soil microbial responses toward the future climate treatment in September. Although microbial biomass showed declining levels in extensive meadows and pastures under future climate treatments, overall, microbial function magnitudes were higher in these land-use types compared to croplands, indicating that improved management practices could sustain high microbial ecosystem functioning in future climates. In contrast to our hypothesis that more disturbed land-use systems would have destabilized microbial functions, intensive meadows and organic croplands showed stabilized soil microbial biomass compared to all other land-use types, suggesting that temporal stability, in addition to magnitude-based measurements, may be useful for revealing context-dependent effects on soil ecosystem functioning.

Key words: aboveground–belowground interactions; community composition; drought; environmental change; functional diversity; land management; soil microorganisms; temporal stability.

INTRODUCTION

Intensive management or land-use practices, including the use of monoculture cropping rotations, tillage, and application of synthetics, continue to threaten long-term soil ecosystem fertility and health across Europe (Foley

et al. 2005, Creamer et al. 2010, EU 2013). Moreover, the impact of both management and climate-change pressures on soil ecosystems pose major scientific challenges in modern ecology research, as climate effects can vary drastically depending on the trajectory and intensity of climate events (i.e., press vs. pulse disturbances; Harris et al. 2018), and their interactions with specific management sub-treatments (“mixed-compounded perturbation”; Kuan et al. 2006, Jurburg et al. 2017). Adapting to and managing the disturbance-response mechanisms of soil microbial communities will be

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imperative for developing resilient and regenerative agriculture practices in future climates, as soil microorganisms play a key role in maintaining soil ecosystem multifunctionality, including carbon sequestration, nitrogen fixation, nutrient provisioning, and organic matter production (van der Heijden et al. 2008, Delgado-Baquerizo et al. 2016, Soliveres et al. 2016, Trivedi et al. 2016).

More attention is being directed toward the development of multi-dimensionality and -functionality metrics for belowground soil processes, which can reveal higher-order trends and synchronizations undetected at the level of individual drivers (Albrich et al. 2018, Barros et al. 2018, Giling et al. 2019). For example, temporal variability is one common proxy indicator of ecosystem stability that can relay and integrate ecosystem response patterns through time (McCann 2000, Proulx 2010, Eisenhauer et al. 2011, Strecker et al. 2016). To date, however, we lack a conceptual understanding of how temporal stability of multiple soil microbial community functions respond to global change and management activities.

Ecosystem stability can be driven by complex species interactions across trophic levels (Dunne et al. 2002, Hedlund et al. 2004, de Ruiter et al. 2005), as well as individual and community-level responses to perturbations (Loreau et al. 2001, Griffiths et al. 2004, Garcia-Raventós et al. 2017). Generally, communities with more genetic diversity have a greater buffering capacity and, thus, increased versatility toward disturbances such as soil pollution (Atlas et al. 1991) and heat (Wertz et al. 2007), leading to increased stability in times of environmental fluctuations and stress (Loreau et al. 2001, Isbell et al. 2015). Stability can also be driven by functional redundancy, whereby coexisting soil microorganisms perform generic functions to maintain equilibrium across different conditions (Griffiths et al. 1997, Bardgett and McAlister 1999, Setälä and McLean 2004, Setälä et al. 2005, Griffiths and Philippot 2013). Moreover, loss of keystone species with highly specialized traits can have stronger adverse impacts on soil ecosystem stability than the loss of functionally generic species (Griffiths et al. 1997).

Multiple disturbance events can lead to strong impairments in the magnitude of soil microbial functions (Griffiths et al. 1997, Liebich et al. 2007), with land management practices exerting significant changes in the provisioning of belowground ecosystem services at local scales (Drenovsky et al. 2010, Dequiedt et al. 2011). However, effects of individual treatments, such as changing plant diversity, on the temporal stability of soil functions remain unclear (Orwin and Wardle 2005, Wagner et al. 2015), and consequently, knowledge on the effects of broader land management systems comprising multiple sub-treatments is critically lacking. To address these knowledge gaps, we test a conceptual framework defining the relationship between land-use, climate change, and temporal stabilities of several commonly

investigated microbial functions as indicators (Fig. 1a). We examine how temporal stability of selected microbial functions, such as basal respiration, microbial biomass, and functional diversity, is influenced by the fungi-to-bacteria biomass ratio, a proxy indicator of community structure and overall ecosystem health (Wardle et al. 2004, Zhang et al. 2005, van der Heijden et al. 2008, Gray et al. 2011). Finally, we assess how land-use and climate-mediated effects on stability–magnitude dynamics for these indicators influence overall ecosystem functioning (Fig. 1b; Griffiths and Philippot 2013, Barros et al. 2018, Albrich et al. 2018).

Climate changes and management practices can interactively destabilize microbial community processes, e.g., by amplifying disruptions to soil water-holding capacity and mycelial networks (Tisdall 1994, Calderón et al. 2000, Liu et al. 2009, Castro et al. 2010, de Vries et al. 2012a, Siebert et al. 2019). However, a recent study conducted at the Global Change Experimental Facility (GCEF) in Bad Lauchstädt, Germany, suggests that warmer and drier climates had a more detrimental effect on microbial communities in extensive sheep pastures (Siebert et al. 2019), indicating that practices associated with more sustainable land management could in fact intensify climate effects, at least in the short term (Siebert et al. 2019, Schädlér et al. 2019). Moreover, climate effects on community structure vary considerably: warm and dry conditions can favor gram-positive bacteria (Gray et al. 2011, Koyama et al. 2018), and significantly impair fungal diversity and biomass (Frey et al. 2008). In contrast, several studies indicate that warmer temperatures promote fungi by enhancing growth rates and litter deposition of plants with high C/N ratios (Zhang et al. 2005, Prieto et al. 2019), and that fungal communities could mitigate drought-induced losses in soil carbon and nitrogen (de Vries et al. 2012a). Other findings show that temperature and precipitation do not strongly influence the fungi-to-bacteria ratio or dominant bacterial and fungal groups therein (Castro et al. 2010, Gray et al. 2011, Li et al. 2017), suggesting context-dependency in microbial community structural responses to climate, as influenced by local management practices, abiotic and biotic conditions (Classen et al. 2015).

In the present study, we hypothesized (1) management to strongly influence microbial communities, with more intensive systems showing reduced microbial function levels. Moreover, we expected (2) fungi-to-bacteria ratios would be strongly influenced by management and unaffected by changes in climate, with higher fungal abundance expected in extensive systems. We also expected (3) the effect of future climate treatments to depend on land-use, resulting in stronger reductions in microbial functions and their stabilities in intensive and conventional cropland systems. (4) Extensive management, as well as organic cropland systems, would show higher temporal stability in soil microbial communities, with future climate treatments having a weaker effect relative to management.

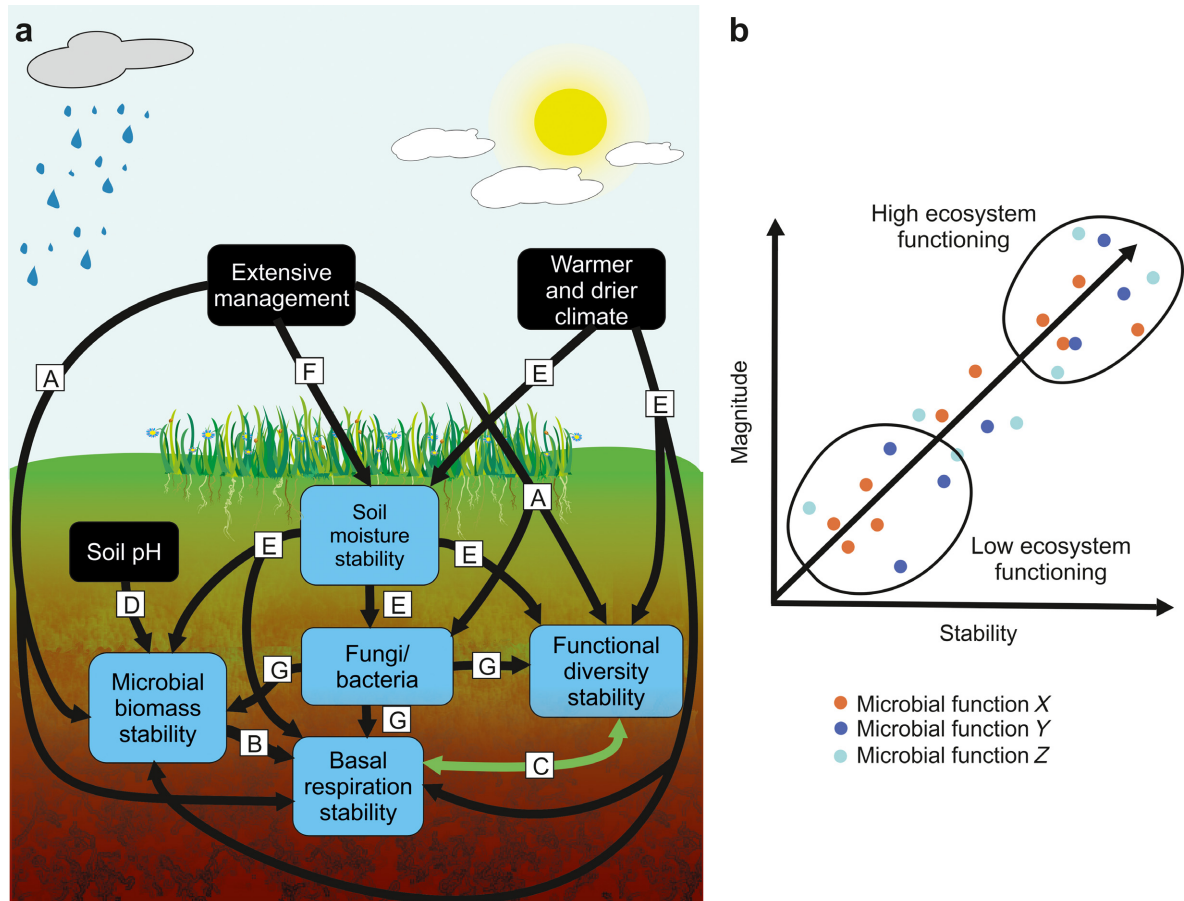


FIG. 1. (a) A priori model with hypothesized links (black arrows) and covariances (green arrow) between global change drivers and temporal stability of soil abiotic and microbial functions. Letters indicate justification of linkages (see Table 1). Boxes indicate exogenous (black) and endogenous (blue) variables. "Extensive management" includes the subset of grassland management systems with high plant diversity and no mechanical tillage or synthetic treatments, with the assumption made that these systems will exhibit functional and stability characteristics that differ greatly from intensive meadows and croplands. (b) Proposed relationship between stability and magnitude of a given microbial function, and the associated level of ecosystem functioning.

MATERIALS AND METHODS

Site description and experimental design

The Global Change Experimental Facility (GCEF) is located on a former agricultural site in Bad Lauchstädt, Germany (51°23'30" N, 11°52'49" E, 116 m above sea level), an area rich in fertile Chernozem soil with high humus content, with mean annual temperature of 8.8°C and precipitation of 484 mm (1896–2003; Altermann et al. 2005). The facility has been in operation since May 2014 and consists of a split-plot design with two climate treatments as the main plot variable and five management systems as the subplot variable, with each management-climate treatment combination replicated five times (Schädler et al. 2019). Management systems include intensive meadows (IM), extensive meadows (EM), extensive sheep-grazed pastures (EP), conventionally farmed croplands (CF), and organically farmed

croplands (OF). In 2018, CF and OF plots received the same mechanical or tillage treatment (i.e., use of harrows, plows, cultivators, and rollers), while inorganic fertilizers were applied regularly in CF and IM plots. CF and OF plots were sown with winter wheat monocultures, and IM plots were sown with a mix of five conventional forage grasses. In EM and EP plots, 56 plant species were sown, including 14 grass species, 10 legumes, and 32 herbs (Schädler et al. 2019). These plant species represent diverse ecological niches in mesophilous to dry meadows and pastures as well as the steppe grasslands of central Germany (Schädler et al. 2019). Detailed sub-treatment schedules can be found in Appendix S1: Tables S1 and S2 and Appendix S2: Figs. S1 and S2.

In future climate blocks, irrigation systems and steel frames with retractable roofs were installed to simulate the climate conditions for central Germany in 2070–2100 (Appendix S2: Figs. S3-S4; Schädler et al.

2019). Temperature was controlled passively by enclosing subplots with roller blinds at night, corresponding to up to 2°–3°C increase at night and a daily mean increase of 0.5°–0.6°C. Precipitation was simulated using roof adjustments and irrigation, corresponding to a 20% reduction in summer (June–August), and 10% increase in spring (March–May) and fall (September–November) relative to natural rainfall (Schädler et al. 2019). In 2018, cumulative annual precipitation (including natural rainfall and treatments) was exceptionally less than previous years: ambient and future climate blocks received 174 and 173 mm of rainfall, compared to 363 and 333 mm in 2017, respectively. Steel frames were installed in ambient climate blocks to account for potential microclimatic effects (Schädler et al. 2019).

Soil sampling and analysis

Samples were collected on 13 March (late winter), 16 May (spring), 12 July (summer), 27 September (early fall), and 7 November (late fall) 2018. Six soil cores (1 cm diameter) from the upper 15 cm soil layer were extracted in each subplot. After root and litter debris were removed, samples were sieved (2 mm) and stored at 4°C, or frozen at –20°C if stored longer than one week, and standard incubation procedures were followed to reestablish microbial communities prior to analyses (see Appendix S3: Supplementary Methods). Soil pH was measured with a Thermo Scientific (Waltham, Massachusetts, USA) Orion Star A211 pH meter after incubating 10 g dry soil in 0.01 mol/L CaCl₂ for 1 h at room temperature (Appendix S2: Fig. S5). Microbial biomass, basal respiration, and metabolic quotient (respiration-to-biomass ratio, used as a proxy indicator for soil metabolic efficiency) were measured within one week of the sampling date according to the protocol from Strecker et al. (2016). As an indicator of functional diversity, carbon substrate utilization (CO₂ evolution rates; μg CO₂–C·g^{–1}·h^{–1}) was measured for 14 substrates using the MicroResp method developed by Campbell et al. (2003), and incorporated into the Shannon diversity index (H'_{mic})

$$H'_{mic} = - \sum_{i=1}^s p_s \log_{10} p_s,$$

where s is the number of substrates, and p_s is the CO₂ rate for each substrate (Morris et al. 2014). Carbon substrates were categorized into amino acids, monosaccharides, carboxylic acids, and disaccharides. Scaled temporal stabilities for functional diversity, microbial biomass, basal respiration, and soil moisture, were calculated using the inverse coefficient of variation (CV^{–1}), or the temporal standard deviation divided by the temporal mean (Proulx et al. 2010, Strecker et al. 2016). Standard deviations and means were compared using linear regression analysis to assess their relative influence on temporal stability, as performed by Robertson et al. (2016).

To assess microbial community structure and fungi-to-bacteria (F/B) ratios, phospholipid and neutral fatty acid analysis (PLFA, NLFA) were performed on samples from May, July, and September according to the protocol from Frostegård et al. (1991). Fatty acid biomarkers were converted to biomasses (ng/g dry soil) and grouped into gram-positive and gram-negative bacteria, general bacteria, arbuscular mycorrhizal (AM) fungi, and saprotrophic fungi (hereafter “other fungi”; Bligh and Dyer 1959, White et al. 1979). Straight-chain saturated fatty acids 14:0, 15:0, 17:0 were used for general bacteria, polyunsaturated fatty acids 18:2ω6c, 18:3ω3, and 18:3ω6c for other fungi, monounsaturated neutral fatty acid 16:1ω5 for AM fungi, terminally branched fatty acids anteiso15:0, iso15:0, iso16:0, and iso17:0 for gram-positive bacteria, and cyclopropyl saturated fatty acids cy17:0 and cy19:0 were used for gram-negative bacteria (Willers et al. 2015). F/B ratio was calculated as the molar mass of fungal biomarkers divided by the sum of molar weights of bacterial biomarkers (Bardgett and McAlister 1999, Zhang et al. 2005, de Vries et al. 2006). To minimize storage effects on soil communities, PLFA and functional diversity measurements were completed within 2–6 weeks of the sampling date (Meyer et al. 2019).

Statistical analysis

Statistical analyses were performed using R software version 3.5.2. Linearized mixed models were built for each response variable using the lme4 package. We employed three-way ANCOVAs to assess effects of land-use, climate and date on microbial functions and carbon substrate class use (Appendix S1: Tables S3 and S4), followed by two-way ANCOVAs separately for each land-use system (using sampling date and climate treatment as fixed effects, and subplot identities as random effects to account for repeated measures) and sampling date (using climate and land-use system as fixed effects, and main plot identities as random effects). Data were tested for normality of residuals and homogeneity of variances using the Shapiro-Wilk and Levene’s test, respectively. If assumptions were violated, data were transformed using Tukey’s ladder of powers transformation, logarithmic transformation, or fit using generalized linear models from the tweedie package with an appropriate distribution. Wherever necessary, temporal autocorrelations were identified by assessing the significance of the linear relationships between microbial functions on consecutive dates. Resulting models were analysed using the car package with type II sum of squares, significance level $\alpha = 0.05$. Pairwise comparisons were performed to determine significant differences ($\alpha = 0.05$) between groups using the multcomp package.

Principal component analyses (PCA) based on the correlation matrix were performed using the factoextra package to assess differences in average substrate class percent CO₂ production and absolute PLFA abundances

TABLE 1. Description of hypothesized linkages between global change drivers and temporal stability of soil abiotic and microbial functions in the a priori model (Fig. 1).

| Link | Description | Source |
|------|--|--|
| A | <p>(1) Unfertilized, undisturbed grasslands and pastures with high plant functional group richness support fungal communities, enhancing ecosystem health and functioning.</p> <p>(2) Absence of fertilizers promotes higher microbial biomass and respiration via alterations to crop biomass, soil organic carbon content and soil pH.</p> <p>(3) Aboveground plant communities shape plant–microbe interactions and abiotic soil properties through altering the quantity and diversity of litter inputs and root exudates.</p> <p>(4) <i>Insurance hypothesis</i>: high plant richness promotes microbial community stability by providing more diverse and continuous inputs of plant-derived resources in space and time and more stabilized abiotic conditions.</p> | Bardgett et al. (1997, 1999), Bardgett and McAlister (1999), Proulx et al. (2010), de Vries et al. (2006, 2012a, b), Lange et al. (2014), Banerjee et al. (2018) |
| B | Microbial biomass is a strong predictor of basal respiration. | Zhang et al. (2005), Strecker et al. (2016), Eisenhauer et al. (2018) |
| C | Basal respiration stability covaries with, but does not predict, functional diversity stability: | Bardgett et al. (1997), Loreau et al. (2001), Lucas et al. (2013), Wang et al. (2017) |
| | (1) Both processes show similar seasonal variations, with declining levels seen between summer and fall. | |
| | (2) <i>Theory of functional equilibrium</i> : decoupling of basal respiration and functional diversity is driven by differential competitive allocation of resources between shoots/roots, as observed when higher plant species richness leads to increasing functional diversity and decreasing basal respiration over time. | |
| | (3) <i>Insurance hypothesis</i> : an adequate supply of non-diverse substrates can still maintain primary productivity (therefore, microbial biomass and substrate quantity drive basal respiration more than the diversity of substrate supply/use). | |
| D | Temporal variability of soil microbial biomass is strongly influenced by soil pH in both arable and grassland ecosystems. | Wardle (1998) |
| E | Climate effects on functional diversity, microbial biomass, and basal respiration are driven by soil moisture and precipitation; climate effects on <i>F/B</i> are generally weak. | Liu et al. (2009), Strickland and Rousk (2010), Li et al. (2017) |
| F | Plant diversity strongly affects soil moisture; absence of tillage improves moisture retention in upper soil layer. | Calderón et al (2000), Lange et al. (2014) |
| G | High <i>F/B</i> promotes increasing microbial biomass stability as driven by a greater abundance of slow-growing fungal communities and symbiotic plant–root associations; fungi facilitate ecosystem functioning and stability via microbial activity and greater tolerance to climatic disturbances. | Bardgett et al. (1997), Zak et al. (2003), Hedlund et al. (2004), Gray et al. (2011), Banerjee et al. (2018) |

in May, July, and September. Structural equation modeling (SEM) was performed to identify relationships between climate treatments and land use on *F/B*, soil abiotic properties, and temporal stability of microbial biomass, basal respiration, and functional diversity using the lavaan and piecewiseSEM packages (Lefcheck 2016). Climate treatment (ambient and future) and land management (intensive management for IM, OF, and CF plots; and extensive management for EM and EP plots) were used as binary exogenous variables. Binary exogenous variables for land management were grouped based on similarities in land-use treatments and validated using separation patterns shown in PCA scores for PLFA biomarker abundance and substrate utilization. We used linear regressions to identify and retain significant a priori hypothesized relationships in the final SEM, and performed model selection and validation with tests of directed separation (Fig. 1, Table 1, Appendix S1: Table S5; Xu et al. 2015, Lefcheck 2016). Goodness-of-fit in the final model was assessed using the chi-square test, Akaike information criterion (AIC), root mean square error of approximation (RMSEA), and comparative fit index (CFI).

RESULTS

Soil microbial functions

Similar shifts in basal respiration, functional diversity, and metabolic quotient in each land-use and climate treatment combination were observed across all five sampling dates, indicating conserved seasonal variability (Fig. 2). Basal respiration and metabolic quotient reflected continuous reductions from March through July, followed by a strong rebound in September, coinciding with higher precipitation levels at this time of year (Appendix S2: Fig. S4). Functional diversity peaked for all land-use systems in July. Basal respiration, microbial biomass, and functional diversity were significantly influenced by land-use for all dates, with elevated values observed in grasslands (EM, IM, and EP) relative to croplands (OF and CF). Microbial biomass exhibited little seasonal variation in intensive meadows and croplands, although increases were observed between March and May for all land-use types (Fig. 2b).

Basal respiration, metabolic quotient, and functional diversity varied due to land-use and climate interactions

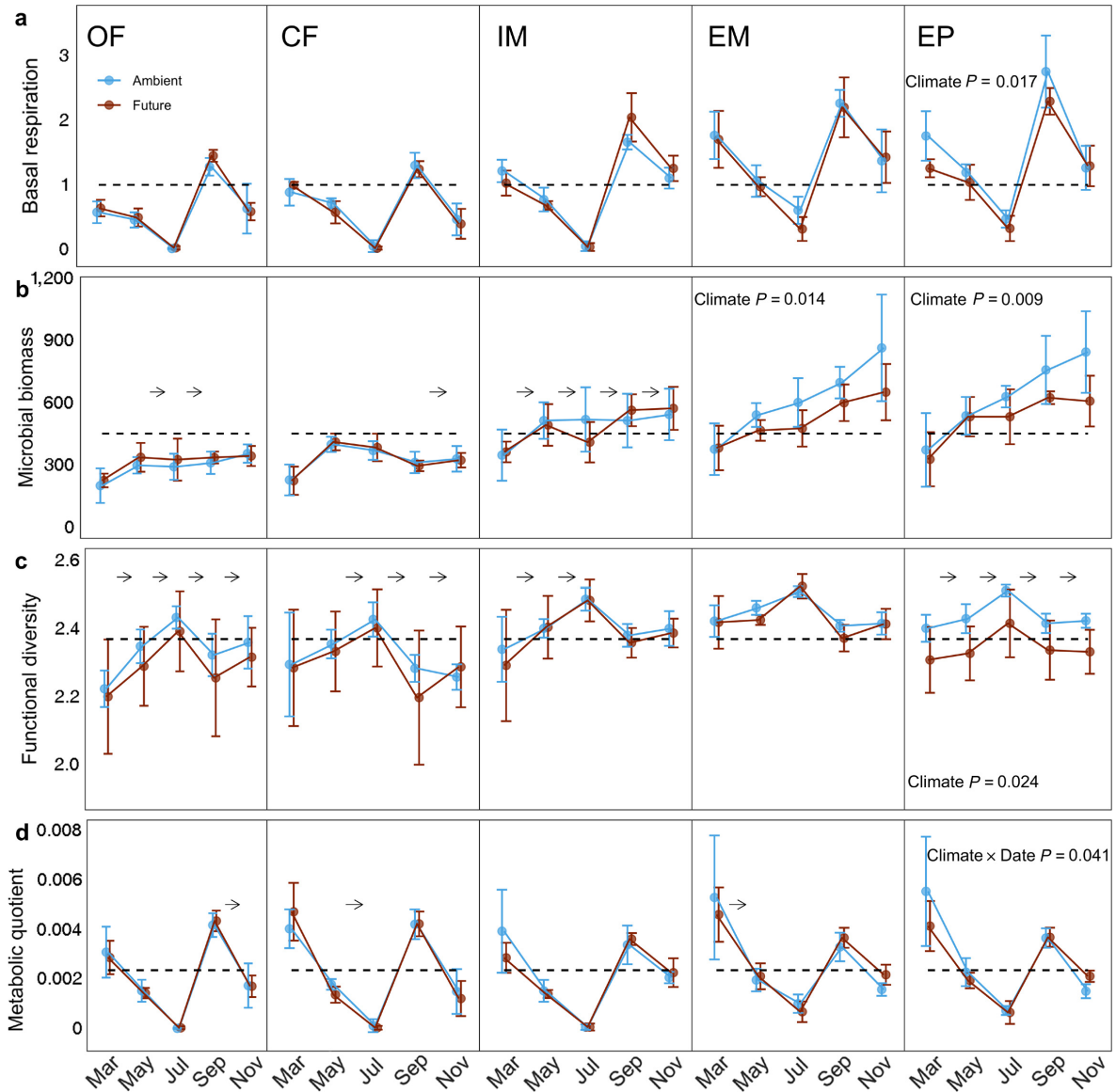


FIG. 2. (a) Basal respiration ($\mu\text{L O}_2 \text{ h}^{-1} \text{ g dry soil}^{-1}$), (b) microbial biomass ($\mu\text{g C}_{\text{mic}} \text{ g dry soil}^{-1}$), (c) functional diversity (unitless, derived from Shannon diversity index, H'_{mic}), and (d) metabolic quotient ($\mu\text{L O}_2 \text{ mg C}_{\text{mic}}^{-1} \text{ h}^{-1}$) for all treatments and sampling dates. Arrows indicate temporal autocorrelations between dates. P values from the two-way ANCOVA within each land type are shown wherever significant climate and interactive effects occurred. Dashed horizontal lines indicate means across all treatments and dates; bars show standard deviation. Columns correspond to individual land systems: CF, conventional croplands; EM, extensive meadows; EP, extensive pastures; IM, intensive meadows; OF, organic croplands.

in September (Table 2). Basal respiration declined in future pastures (−16.8%) and increased in future intensive meadows (+23.2%; Fig. 2a). Functional diversity declined in future pastures (−3.3%), organic (−2.9%), and conventional croplands (−3.8%), while the metabolic quotient increased in future intensive (+7.0%) and extensive meadows (+11.2%), indicating low carbon-use efficiency (Fig. 2c and d). Land-use and climate interactions influenced microbial biomass in extensive pastures and meadows: biomass levels increased steadily in both

management systems under ambient conditions between July and November, but these changes were considerably reduced in future climate treatments (Fig. 2b). Climate-driven reductions in microbial biomass were most pronounced in future extensive meadows (−24.7%) and pastures (−28.1%) in November. Moreover, future climate effects were the strongest in extensive pastures compared to other land-use types, yielding substantial reductions in basal respiration and functional diversity throughout the year. Significant date and climate interactions also

TABLE 2. ANCOVA table of F values for effects of land use (Land), climate, and covariates on basal respiration (BR), microbial biomass (C_{mic}), functional diversity (H'), and metabolic quotient (qO_2) for each date.

| Response and predictor | df _{Num} , df _{Den} | Mar | May | Jul | Sep | Nov |
|-----------------------------|---------------------------------------|-----------------|-----------------|----------------|-----------------|-----------------|
| BR | | | | | | |
| Land | 4, 30 | 20.42*** | 11.29*** | 9.82*** | 45.03*** | 17.13*** |
| Climate | 1, 8 | 0.41 | 0.05 | 1.77 | 0.12 | 0.02 |
| Land × Climate | 4, 30 | 1.79 | 1.83 | 0.55 | 2.66* | 0.26 |
| Soil moisture | 1, 30 | 6.41* | 10.32** | 0.05 | 1.03 | 0.96 |
| pH | 1, 30 | 0.51 | 0.00 | 0.06 | 1.25 | 0.17 |
| C_{mic} | | | | | | |
| Land | 4, 30 | 11.77*** | 25.99*** | 4.79** | 90.74*** | 34.52*** |
| Climate | 1, 8 | 2.81 | 0.28 | 3.68 | 3.28 | 5.49* |
| Land × Climate | 4, 30 | 0.96 | 3.79* | 2.16 | 3.00* | 2.95* |
| Soil moisture | 1, 30 | 7.08* | 9.41** | 2.09 | 5.50* | 2.44 |
| pH | 1, 30 | 14.98** | 26.85*** | 17.82** | 15.94*** | 11.63** |
| H' | | | | | | |
| Land | 4, 30 | 5.71** | 8.67*** | 3.70* | 20.75*** | 7.63*** |
| Climate | 1, 8 | 0.08 | 0.18 | 0.31 | 1.97 | 0.02 |
| Land × Climate | 4, 30 | 0.56 | 2.37 | 1.71 | 3.01* | 1.45 |
| Soil moisture | 1, 30 | 0.00 | 3.33 | 0.02 | 4.53* | 0.53 |
| pH | 1, 30 | 14.18** | 11.04** | 3.14 | 20.02*** | 8.55* |
| qO_2 | | | | | | |
| Land | 4, 30 | 1.00 | 1.44 | 7.10*** | 11.59*** | 2.25 |
| Climate | 1, 8 | 0.05 | 0.08 | 0.16 | 4.53* | 3.06 |
| Land × Climate | 4, 30 | 0.73 | 0.50 | 0.51 | 0.24 | 2.09 |
| Soil moisture | 1, 30 | 12.50** | 0.46 | 0.02 | 0.49 | 0.14 |
| pH | 1, 30 | 10.38** | 10.16** | 1.08 | 10.68** | 5.95* |

Notes: F values are in boldface type when significant ($\alpha = 0.05$) and marked with asterisks corresponding to the level of significance; df_{Den} denotes degrees of freedom of the denominator; df_{Num} denotes degrees of freedom of the numerator.
 *** $P < 0.001$, ** $0.001 < P < 0.01$, * $0.01 < P < 0.05$

led to pronounced changes in the metabolic quotient in extensive pastures, with future climate treatments showing a reduction in the metabolic quotient in March (−25.3%), and increasing relative to ambient climate treatments in November (+40.0%).

MicroResp carbon substrate utilization data revealed similar management-specific clustering patterns for all dates, with organic and conventional croplands clearly separated from extensive meadows and pastures (Fig. 3a and b). Intensive meadows were somewhat distinct from other land-use types in July, but grouped more closely to extensive meadows and pastures in May and September. The first two principal components accounted for 64.5%–85.2% (PC1) and 8.6%–23.2% (PC2) of total variance, with PC1 accounting for the separation between croplands and grasslands. Variations in amino acid, monosaccharide, disaccharide, and H₂O use had a similar effect, as observed by their relatively equal contributions to PC1 (21.3%–29.1%, 22.2%–28.1%, 9.0%–25.7%, and 19.8%–26.5%, respectively), while carboxylic acid use had a distinct effect, showing a much higher contribution to PC2 (52.0%–81.0%).

Soil microbial community structure

There was no effect of climate on F/B ($P = 0.599$), but land-use interacted significantly with sampling date

($P = 0.014$) and led to the highest F/B across all systems in May. F/B decreased strongly from May to July in all land-use systems, and showed the least variability from May through September in croplands and intensive meadows (Appendix S2: Fig. S6A and B). F/B was largely driven by AM fungal abundance, which was greatest in extensive meadows and pastures in May and July (Appendix S2: Fig. S6A and B). In May, AM fungi accounted for 45%–52% of total PLFA biomass in extensive meadows, 30%–57% in extensive pastures, 18% in organic croplands, 7%–13% in intensive meadows, and 4%–6% in conventional croplands (Appendix S2: Fig. S6B). In extensive meadows and pastures, and to a lesser extent in intensive meadows, AM fungal abundance decreased steadily from May to September, corresponding to a reduction in F/B , while other fungi and gram-negative bacteria increased. In extensive meadows and pastures, gram-positive bacteria declined from May to July then increased rapidly from July to September, while in intensive meadows, gram-positive bacteria increased steadily across all months. F/B rebounded strongly in organic croplands in September, and was attributed to a strong increase in AM fungal abundance after July (Appendix S2: Fig. S6A). Changes in microbial group composition across land-use systems and dates were consistent in both relative and absolute abundance analyses.

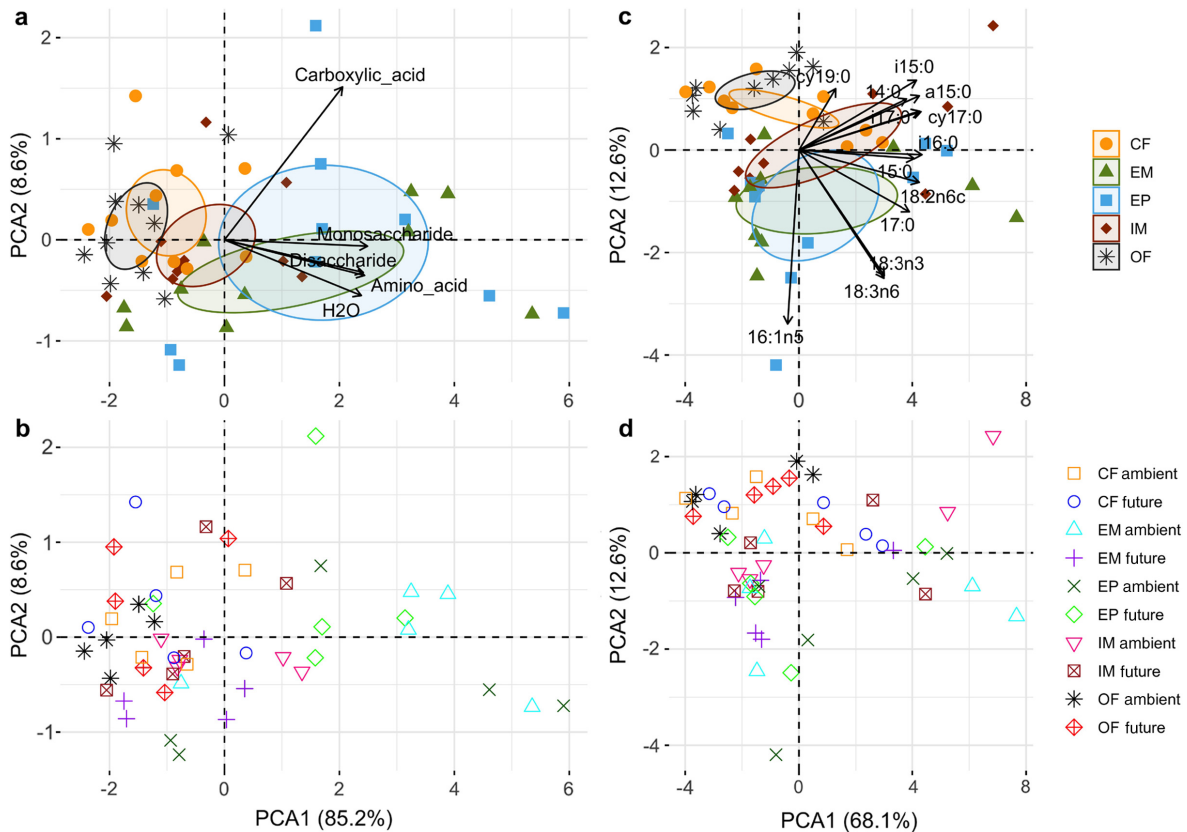


FIG. 3. July PCA biplot for MicroResp substrate class utilization patterns showing (a) land-use-specific treatments and (b) scores for all land use and climate treatment combinations. July PCA biplot for PLFA biomarker abundances showing (c) land-use-specific treatments and (d) scores for all land use and climate treatment combinations. CF, conventional croplands; EM, extensive meadows; EP, extensive pastures; IM, intensive meadows; OF, organic croplands.

TABLE 3. ANCOVA table of F and P values for effects of land use (Land) and climate on the temporal stability of basal respiration (BR), microbial biomass (C_{mic}), and functional diversity (H').

| Predictor | df _{Num} , df _{Den} | BR | | C_{mic} | | H' | |
|----------------|---------------------------------------|-------------|---------------------|-------------|---------------|-------------|---------------|
| | | F | P | F | P | F | P |
| Land | 4, 30 | 8.95 | <0.001*** | 3.91 | 0.011* | 2.79 | 0.043* |
| Climate | 1, 8 | 0.47 | 0.511 | 0.64 | 0.443 | 0.75 | 0.405 |
| Land × Climate | 4, 30 | 1.12 | 0.364 | 1.18 | 0.338 | 0.46 | 0.764 |
| Soil moisture | 1, 30 | 7.22 | 0.011* | 0.04 | 0.849 | 0.00 | 0.963 |
| pH | 1, 30 | 0.03 | 0.872 | 4.79 | 0.035* | 0.15 | 0.699 |

Notes: Data for H' and C_{mic} were transformed using Tukey's ladder of powers transformation. F and P values are denoted in boldface type when significant ($P < 0.05$) and marked with asterisks corresponding to the level of significance; df_{Den} denotes degrees of freedom of the denominator; df_{Num} denotes degrees of freedom of the numerator.

*** $P < 0.001$, ** $0.001 < P < 0.01$, * $0.01 < P < 0.05$

PLFA biomarker abundances clearly distinguished between croplands and grasslands for all dates, indicating that major land-use changes, but not climate, accounted for shifts in community structure (Fig. 3c and d). Intensive meadows were somewhat distinct from other land-use types in July, but showed more similarity to croplands in May, and grouped closely with extensive meadows and pastures in September. The first principal component (PC1) accounted for 53.8%–68.1% of the

variation due to lower PLFA abundances in the croplands. The second axis accounted for 12.1%–12.6% (PC2) of total variance; mainly due to higher AM fungal abundance (biomarker 16:1ω5) in grasslands.

Temporal stability of soil microbial functions

Land use, but not climate, affected the temporal stability of microbial functions (Table 3). Basal respiration

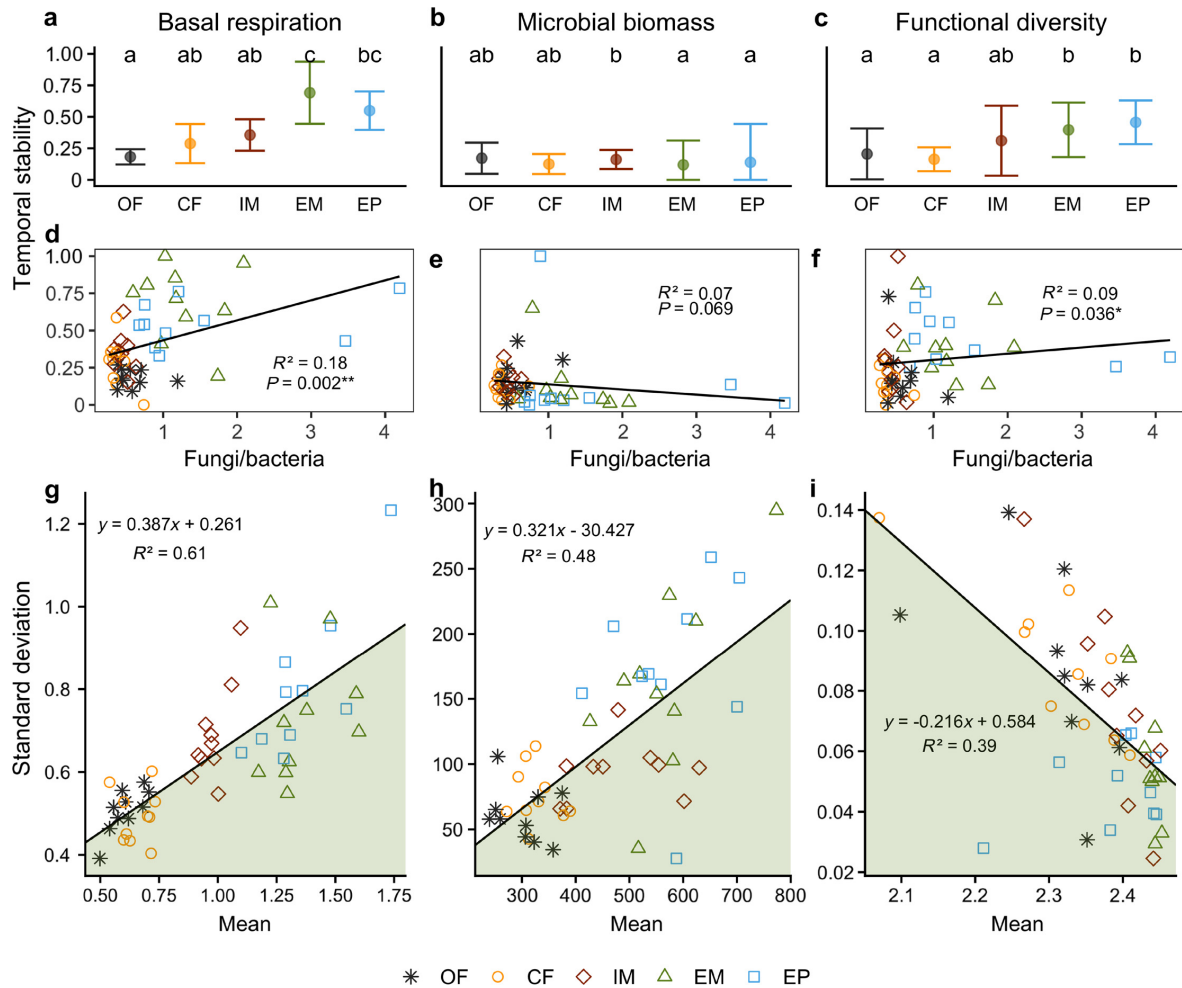


FIG. 4. (a–c) Temporal stability (unitless, scaled between 0–1) grouped by land-use type for (a) basal respiration, (b) microbial biomass, and (c) functional diversity. Letters indicate significant differences of means for each land-use system. (d–f) Relationships between the temporal stability of (d) basal respiration, (e) microbial biomass, and (f) functional diversity and the fungi-to-bacteria ratio. Fungi-to-bacteria ratios were calculated from the average of the three dates in each subplot. (g–i) Plots of temporal means vs. temporal standard deviations for (g) basal respiration, (h) microbial biomass, and (i) functional diversity. Coefficient of determination (R^2) and linear regression equations are shown. Shaded regions highlight where the response scores fall below the regression line, indicating a higher relative influence of the temporal mean on the temporal stability, compared to temporal standard deviation. CF, conventional croplands; EM, extensive meadows; EP, extensive pastures; IM, intensive meadows; OF, organic croplands.

stability was greater in extensive meadows and pastures than in the other land-use types (Fig. 4a), while microbial biomass stability was lower in conventional croplands, extensive meadows and pastures, reflecting more variation due to changing microbial biomass levels throughout the year (Figs. 2b and 4b). Functional diversity stability was greater in the grasslands than croplands (Fig. 4c). *F/B* ratios correlated positively with basal respiration and functional diversity stability (Fig. 4d and f), but were not associated with microbial biomass stability (Fig. 4e).

Standard deviations and means correlated positively for basal respiration and microbial biomass, and negatively for functional diversity (Fig. 4g–i). For basal respiration and functional diversity stability, greater

temporal stability seen in extensive meadows and pastures was attributed to higher means relative to standard deviations (Fig. 4g and i). In contrast, reduced microbial biomass stability seen in these systems was attributed to higher standard deviations relative to means (Fig. 4h).

Extensive management land types (EM and EP) had a direct and positive effect on the *F/B* ratio and temporal stability of basal respiration and functional diversity (Fig. 5a). Furthermore, temporal stability of basal respiration was indirectly mediated by changes in soil moisture (Fig. 5b). Here, extensive management increased soil moisture, thereby stabilizing microbial basal respiration, whereas future climates appeared to counteract this effect by promoting soil moisture loss.

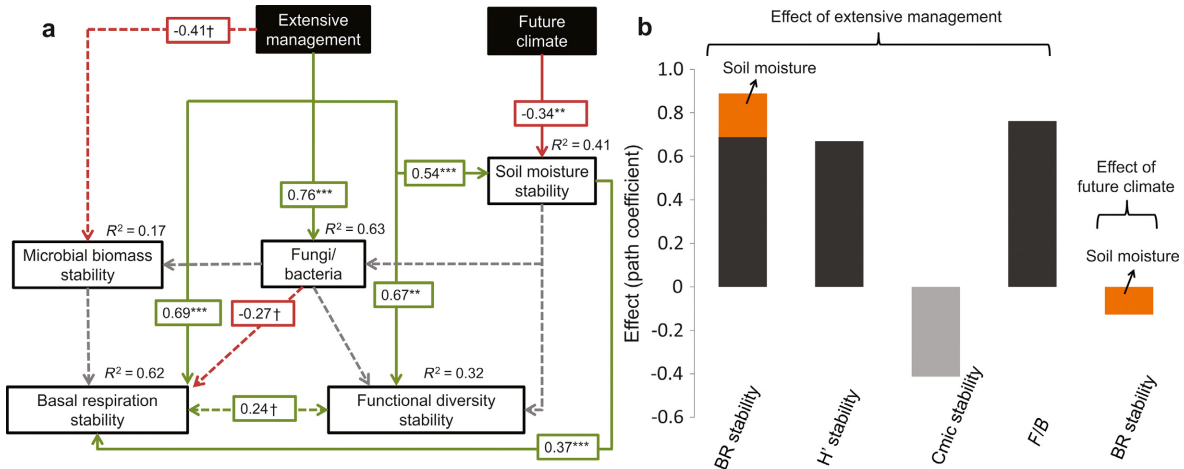


FIG. 5. (a) Structural equation model ($df = 12$) relating climate treatment, extensive management (EM and EP plots) and F/B ratio to temporal stability of soil microbial functions ($n = 50$). Numbers along arrows indicate standardized path coefficients and level of significance ($***P < 0.001$, $**0.001 < P < 0.01$, $*0.01 < P < 0.05$, $†0.05 < P < 0.10$). Boxes indicate exogenous (black) and endogenous variables (white); lines indicate non-significant (dashed and faded), marginally significant (dashed, $0.05 < P < 0.10$), or significant links (solid, $P < 0.05$); arrows represent direct causal paths (single-headed) and covariates (double-headed); green and red indicates a positive or negative relationship, respectively. Fungi-to-bacteria ratio was calculated using the average for May, July, and September. Proportion of variance explained (R^2) is shown next to the response variables. Goodness of fit measures: $\chi^2 = 5.886$; $P = 0.922$; CFI = 1.000; AIC = -529.464; RMSEA = 0.000, $P = 0.951$. (b) Total direct and indirect effects on temporal stability of basal respiration (BR), functional diversity (H), microbial biomass (C_{mic}), and fungi-to-bacteria ratio (F/B), indicated by the standardized path coefficient. Indirect effects were calculated by multiplying significant path coefficients between each variable. Significant effects are indicated in dark gray (direct) and orange (indirect); marginally significant direct effects ($0.05 < P < 0.010$) are light gray.

DISCUSSION

Climate effects and magnitude of microbial functions driven by land-use type

Our study provides strong evidence to support that land management intensification is a significant driver of change in microbial communities (Drenovsky et al. 2010, Dequiedt et al. 2011). Declining levels of basal respiration, microbial biomass, and functional diversity observed in intensive meadows and especially croplands suggested decreased ecosystem functioning through inhibitory mechanisms driven by land-use specific sub-treatments including tillage, mowing, synthetic fertilizers, and cropping patterns. In intensive meadows and croplands, reductions in microbial biomass and basal respiration were attributed to losses in root biomass and root C inputs induced by N fertilizer application (Lovell et al. 1995). Absence of tillage in extensive meadows and pastures would have preserved intact fungal mycelia, soil structural integrity and aeration in these soils (Le Guillou et al. 2018), accounting for the high levels of functioning seen across microbial indicators. Furthermore, higher plant species richness in these systems would have promoted microbial biomass and activity in the grasslands through increasing the quantity (Zak et al. 2003, Eisenhauer et al. 2010, Lange et al. 2014) and diversity of soil rhizodeposits (Lange et al. 2015, Eisenhauer et al. 2017).

Likewise, greater diversity in plant-specific root functions and morphological traits, such as biomass and root depth, may have helped to sustain a wider range of specialized plant–soil feedbacks, thus enhancing ecosystem processes and rhizosphere activity (Wolters et al. 2000, Loreau et al. 2001, Eisenhauer et al. 2018).

Similar response magnitudes in organic and conventional croplands were unexpected, as organic land-use systems typically show higher levels of functioning and can more readily metabolize complex substrates (Martinez-Garcia et al. 2018). However, a recent study by Le Guillou et al. (2018) showed that mechanical disturbance, pre-existing soil organic carbon content, and the C/N ratio of the upper soil layer are far more important drivers of microbial communities than management-based changes to organic inputs and crop residues. We suspect that tillage treatment in organic croplands may have counteracted the beneficial effects of no synthetic N fertilizer application, leading to similar reductions in microbial biomass and activity in both croplands. Additionally, despite reincorporation of crop residues into the soil after harvesting in both croplands, microbial function levels were less than in grasslands, indicating that crop residue inputs did not compensate for losses in ecosystem functioning in the presence of tillage. We call for more studies on long-term effects, given that effects of land-use change on soil microorganisms may need multiple years to materialize (Habekost et al. 2008, Eisenhauer et al. 2010).

Fungi-to-bacteria ratios driven by land-use type and not strongly influenced by climate

Higher fungi-to-bacteria ratios observed in extensive meadows, pastures, and organic croplands were in line with previous studies (Drenovsky et al. 2010, de Vries et al. 2006, Martinez-Garcia et al. 2018). Plant biomass removal in the intensive meadows, along with frequent tillage in the croplands, likely inhibited fungal community growth by reducing litter inputs and destroying mycelia structure (Tisdall 1994, Calderón et al. 2000, Zhang et al. 2005). Likewise, N fertilizer application in intensive meadows and conventional croplands would have created unfavorable conditions for fungal communities, which thrive in grasslands with N-conservative plant communities and high C/N ratios (de Vries et al. 2012b). Additionally, greater plant diversity in the extensive meadows and pastures likely increased soil productivity and nutrient acquisition, thereby promoting hyphae activity and AM fungal communities (van der Heijden et al. 1998, Scherber et al. 2010, Lange et al. 2014, Eisenhauer et al. 2017).

AM fungi-driven reductions in *F/B* were not affected by GCEF climate treatments during our monitoring time frame, in agreement with previous findings showing that the *F/B* ratio, and fungal communities in general, can be tolerant to precipitation and temperature changes (Castro et al. 2010, Gray et al. 2011, Zhang et al. 2016, Li et al. 2017). This may be attributed to the lower dispersal and turnover rates of AM fungi compared to bacteria (Hedlund et al. 2004), and the higher relative vulnerability of symbiotic fungi-root associations to physical disturbances (Tisdall 1994). However, management systems with high *F/B* ratio in our study were the most affected by future climate treatments, indicating that *F/B* ratios and biomass indicators in general may not reflect complementary traits, functional redundancies, and dynamic changes (i.e., growth rates) that drive community responses to climatic disturbances (Rousk et al. 2009, Strickland and Rousk 2010, van Groenigen et al. 2010).

Climate effects on microbial functions dominated by drought

In September, a month where rainfall levels in both ambient and future climate treatments more than doubled that of the previous two months combined, we observed (1) significant increases in basal respiration and metabolic quotient, and declining functional diversity levels for all land-use systems, and (2) significant interactions between land-use and climate treatments, driven by stronger climate effects in extensive pastures. Though seasonal effects could not be ruled out, we suspect that GCEF climate treatments and the actual drought conditions in central Germany interactively affected microbial communities at this time (GCEF rainfall was 50% below that of 2017 in both climate

treatment blocks; lowest consecutive rainfall levels occurred between May-August, see Appendix S2: Fig. S4). Microbial communities were likely more susceptible to climate treatments (press disturbance) after the drought (pulse disturbance), due to amplified losses in soil moisture and plant cover (Liu et al. 2009, Castro et al. 2010, Manzoni et al. 2012, Evans and Wallenstein 2012, de Vries et al. 2012b). As well, peak functional diversity levels were seen in July, despite extremely low rainfall during the month (9.1 mm in ambient climate blocks, 2.46 mm in future climate blocks). Here, stress-induced physiological adaptations in microbial communities may have promoted increasing versatility toward substrate use, as was demonstrated in a recent study showing a strong negative association between functional diversity and soil moisture (Li et al. 2017). Consequently, declining substrate-use diversity in September was attributed to both higher precipitation levels during this month as well as conserved autumn seasonal effects (Lucas et al. 2013).

The absence of significant climate effects for basal respiration and functional diversity on most other sampling dates points to the high seasonal variability of the region, which may promote greater physiological tolerance of microbial functions toward press-disturbances (Bardgett et al. 1997, Waldrop and Firestone 2006). Furthermore, as soil detritivore abundance and feeding activity are known to mediate soil microbial responses to climate change (Gray et al. 2011, Thakur et al. 2018), ecosystem processes at higher trophic levels may have dampened climate effects. In contrast, microbial biomass levels were affected by climate and land-use interactions throughout the year. This may be indicative of synergistic or additive responses to multiple perturbations (Griffiths et al. 1997), as well as delayed responses to past disturbances (Evans and Wallenstein 2012, Li et al. 2017). Additionally, warm and dry conditions are known to more strongly affect microbial abundance as compared to other microbial functions, suggesting that the multiple climate drivers in our study had a pronounced effect on microbial biomass (Gray et al. 2011, Thakur et al. 2018).

Climate effects more detrimental in extensive meadows and pastures

Significant impairments appeared in the future climate treatments of extensive pastures, and to a lesser extent, extensive meadows, primarily through their effects on microbial biomass. Our results reflect findings reported in the previous year for extensive pastures (Siebert et al. 2019), and suggest that drought-intolerant plant species in these systems reinforced adverse climate effects through losses in vegetation cover (Siebert et al. 2019), resource depletion (Wright et al. 2015), and impaired plant-microbe interactions (Zhang et al. 2005, Sheik et al. 2011, Gray et al. 2011). Furthermore, climate-induced changes in grazing activity in the extensive

pastures may have negatively affected microbial communities due to changes in enzyme activity (Hewins et al. 2015). Nonetheless, these land-use systems exhibited substantially higher microbial biomass and function levels than the intensive meadows and croplands in future climates, suggesting that management practices incorporating higher plant diversity can buffer microbial communities from severe losses in levels of functioning (de Vries et al. 2012a, Isbell et al. 2015).

Non-uniform differences in temporal stability of microbial functions across land-use systems

Temporal stability of microbial functions was driven by land-use, but not climate treatments. Basal respiration and functional diversity stability were greatest in extensive meadows and pastures, followed by intensive meadows, corroborating evidence that management systems with fewer perturbations and high plant richness may stabilize microbial functions (Wagner et al. 2015) by enhancing redundancy and functional complementarity in plant–microbe interactions (Loreau et al. 2001, Proulx et al. 2010). These conditions likely deteriorated in the intensive meadows and croplands, leading to lower magnitudes and temporal stabilities. Moreover, structural equation modeling revealed that management decisions strongly influenced changes in the F/B ratio and temporal stabilities of microbial functions. However, F/B alone did not predict temporal stabilities of microbial functions in our model, despite evidence that AM fungi (a significant driver of F/B in our study) are a keystone microbial group that promote favorable soil conditions and high microbial activity levels in undisturbed soil ecosystems (Griffiths et al. 1997, Banerjee et al. 2018). Interestingly, the apparent decoupling of F/B from functional diversity stability corroborates previous studies assessing the effects of community structure on multi-substrate utilization levels (Griffiths et al. 1997, Grayston et al. 2001, Colombo 2015, Powell et al. 2015), and suggests that while AM fungal abundance is an essential predictor of ecosystem functioning, other dynamic processes, such as soil enzyme activity (Moscatelli et al. 2018) or N availability (Koranda et al. 2014) may drive stability.

The absence of significant climate treatment effects on temporal stability was partly attributed to natural seasonal variations throughout the sampling period, which may have overshadowed subtler treatment-induced effects. However, we note that despite ongoing precipitation treatment in 2018 (decreased summer precipitation, increased spring and autumn precipitation in future climate relative to ambient climate), total mean rainfall levels differed by only 1 mm between treatments, compared to 30 mm in 2017. This was attributed to the lower rainfall levels caused by the drought, and raises concerns that the future climate treatment may have been weakened compared to non-drought years. Though we did not assess the effects of drought conditions on the

climate treatment, we suspect that long-term field experiments may be especially vulnerable to unforeseen climate events that could impact the magnitude and reliability of controlled climate treatments. Nevertheless, multi-year monitoring studies at the same field experiments will provide opportunities to fill critical knowledge gaps on press-and-pulse climate disturbances and their interactive effects on soil microbial communities.

Taken together, the lower magnitudes yet higher stability of microbial biomass in organic croplands and intensive meadows suggested impaired ecosystem functioning relative to extensive meadows and pastures. Frequent mechanical and synthetic treatments alongside low plant diversity likely favored the establishment of homogenous microclimatic conditions that were partly shielded from climatic perturbations, thereby suppressing normal biomass cycles, lowering responsiveness and increasing stability (Wolters et al. 2000). Apart from the conventional croplands, which showed both reduced and destabilized biomass, these results contest the theory that intensively managed land types are destabilized by climate disturbances (Kuan et al. 2006, Waldrop and Firestone 2006). Moreover, though biomass levels were highest in extensive meadows and pastures, their temporal instability suggests they may have “higher to fall” (Pfisterer and Schmid 2002, Wright et al. 2015). On the other hand, microbial biomass instability in extensive meadows and pastures corresponded to changing biomass levels throughout the year, potentially indicating a seasonal shift or delayed recovery to historical perturbations.

Climate treatments at long-term field experiments may be less significant drivers of long-term legacy effects (Rousk et al. 2013), however, management practices can be detected several years after application (Martinez-Garcia et al. 2018). Legacy management effects can influence long-term C_{org} and vary depending on abiotic soil conditions (Geisseler and Scow 2014), suggesting that specific land-use systems at field experiments may be more susceptible to historical treatments than others. For instance, microbial biomass responses to long-term fertilization treatments are driven by soil pH, with acidic soils showing a strong increase in biomass levels in fertilized systems (Geisseler and Scow 2014). Potential impacts of historical management practices on microbial function dynamics can be minimized by homogenizing soil conditions with the use of cover crops, as was done at all GCEF subplots in 2013, before implementing the experimental treatments (Schädler et al. 2019). Additionally, the use of unmodified field controls along with multi-year monitoring of stability-magnitude dynamics would be needed to fully account for legacy effects at different field experiments, particularly if these areas were under intensive agriculture management in the past.

Although standard sieving and storage/freezing procedures can preserve functions, such as microbial respiration, for up to seven weeks (Meyer et al. 2019), modifications and stricter measurement timelines may

be necessary to limit confounding effects of storage on other soil functions and their temporal dynamics. In this study, longer storage times prior to Microresp experiments may have influenced functional diversity measurements through driving metabolic shifts toward carboxylic acids and recalcitrant organic matter (Goberna et al. 2005). Minimization of storage time before analyses, as well as adjustment of storage and incubation temperatures to reflect the seasonal climate of the samples, may help to enhance the reliability and reproducibility of these assessments.

Temporal stability and other multidimensional metrics, such as spatial stability, remain valuable for understanding context-specific responses of microbial communities across a range of disturbances (Bardgett et al. 1997, Kuan et al. 2006, Bond-Lamberty and Thomson 2010, Strecker et al. 2016). However, our results show that combined stability indices derived from multiple microbial community functions should be used with caution to infer ecosystem functioning, as individual functions may exhibit distinct stability patterns that cannot be interpreted when integrated with others (Wright et al. 2016). Temporal stability is highly context dependent: increasing stability might indicate enhanced ecosystem functioning when greater functional complementarity produces an insurance effect (Proulx et al. 2010), while in other cases, declines could also imply higher ecosystem functioning in communities with greater phenotypic plasticity adapting to environmental stressors (Wright et al. 2015, 2016). Ultimately, synchronized measurements of stability and magnitude across a diverse set of indicators (i.e. “multistability” analyses) are needed for assessing complex changes in microbial communities.

CONCLUSIONS

Our study highlights how individual sub-treatments that constitute a given management system play a critical role in modulating soil ecosystem functioning. Furthermore, we show how multidimensional approaches can reveal stability trade-offs that would complement magnitude-based assessments of ecosystem multifunctionality. Though our results are specific to one field experiment, they provide a basis for interpreting climate effects on key ecosystem processes in multi-use land systems that may be generalizable to other geographic regions. However, coordinated efforts to monitor and integrate long-term temporal dynamics of soil microbial communities across diverse sites and ecosystems are needed to reveal the context specificity of ecosystem processes. Moreover, the GCEF climate simulations and drought conditions in 2018 provided an opportunity to investigate microbial processes under heterogeneous climatic perturbations that are rapidly becoming the new normal. Future studies should continue to investigate how even the most fundamental ecosystem processes might evolve against an onset of heterogeneous pulse-

and-press climate events. In this context, long-term monitoring studies will remain highly valuable as a source of real-time data that can contribute to the development of sustainable land-management policies and improved predictive models in a changing world.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.2325/full>

DATA AVAILABILITY

Data (Kostin et al. 2021) are available in the iDiv Data Repository at: <https://doi.org/10.25829/ividiv.1888-24-4321>.