New Phytologist

Research

Drought resistance and resilience of rhizosphere communities in forest soils from the cellular to ecosystem scale – insights from ^{13}C pulse labeling

Decai Gao[1,2,3]*, Jörg Luster[1], Alois Zürcher[1], Matthias Arend[1,4], Edith Bai[5], Arthur Gessler[1,6], Andreas Rigling[1,6], Marcus Schaub[1], Martin Hartmann[7], Roland A. Werner[8], Jobin Joseph[1], Christian Poll[9] and Frank Hagedorn[1]*

[1]Swiss Federal Institute for Forest, Snow and Landscape Research, 8903, Birmensdorf, Switzerland; [2]Qianyanzhou Ecological Research Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, 100101, Beijing, China; [3]College of Resources and Environment, University of Chinese Academy of Sciences, 100190, Beijing, China; [4]Physiological Plant Ecology, University of Basel, 4056, Basel, Switzerland; [5]Key Laboratory of Geographical Processes and Ecological Security of Changbai Mountains, Ministry of Education, Northeast Normal University, 130024, Changchun, China; [6]Terrestrial Ecosystems, ETH Zürich, 8092, Zürich, Switzerland; [7]Sustainable Agroecosystems Group, Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, 8092, Zürich, Switzerland; [8]Agricultural Sciences, ETH Zürich, 8092, Zürich, Switzerland; [9]Soil Biology, University of Hohenheim, 70599, Stuttgart, Germany

Author for correspondence:
Frank Hagedorn
Email: frank.hagedorn@wsl.ch

Received: *1 December 2023*
Accepted: *6 February 2024*

New Phytologist (2024)
doi: 10.1111/nph.19612

Key words: carbon allocation, drought, forest, isotope tracing, microbial community, PLFA, rhizosphere.

Summary

- The link between above- and belowground communities is a key uncertainty in drought and rewetting effects on forest carbon (C) cycle.
- In young beech model ecosystems and mature naturally dry pine forest exposed to 15-yr-long irrigation, we performed ^{13}C pulse labeling experiments, one during drought and one 2 wk after rewetting, tracing tree assimilates into rhizosphere communities.
- The ^{13}C pulses applied in tree crowns reached soil microbial communities of the young and mature forests one and 4 d later, respectively. Drought decreased the transfer of labeled assimilates relative to the irrigation treatment. The ^{13}C label in phospholipid fatty acids (PLFAs) indicated greater drought reduction of assimilate incorporation by fungi (−85%) than by gram-positive (−43%) and gram-negative bacteria (−58%).
- ^{13}C label incorporation was more strongly reduced for PLFAs (cell membrane) than for microbial cytoplasm extracted by chloroform. This suggests that fresh rhizodeposits are predominantly used for osmoregulation or storage under drought, at the expense of new cell formation. Two weeks after rewetting, ^{13}C enrichment in PLFAs was greater in previously dry than in continuously moist soils. Drought and rewetting effects were greater in beech systems than in pine forest.
- Belowground C allocation and rhizosphere communities are highly resilient to drought.

Introduction

Extreme climatic events, such as an enhanced frequency and intensity of drought or intermittent heavy rainfall, are predicted to increase in Central Europe (Gobiet *et al.*, 2014; Stocker, 2014). The impacts of drought on microbial communities range from immediate reductions in metabolic activities, to acclimation of physiological processes, to shifts in species composition under reoccurring drought (Hagedorn *et al.*, 2016; Hartmann *et al.*, 2017; Schimel, 2018). In general, fungi have been observed to be rather resistant to drought compared with bacteria (Yuste *et al.*, 2011; de Vries *et al.*, 2018; Sun *et al.*, 2020; Wilhelm *et al.*, 2023). However, gram-positive bacteria with a thick peptidoglycan cell membrane layer can be even more resistant to natural drought than fungi (Fuchslueger *et al.*, 2016; Hartmann *et al.*, 2017). In addition to the resistance of microorganisms, their resilience, that is their ability to recover by reaching the same activity level as before drought, is of central importance in how microbial communities cope with drought (Hodgson *et al.*, 2015; Ingrisch *et al.*, 2020; Canarini *et al.*, 2021). The increased cell repair and maintenance costs required to resume microbial growth upon drought recovery may lead to drought legacy effects on microbial metabolism (Brangarí *et al.*, 2020).

As above- and belowground systems are tightly coupled, the responses of microbial communities to drought are particularly complex because drought effects on microbial communities colonizing the rhizosphere are linked to plant responses (Solly *et al.*, 2023). More than half of recent tree photosynthates are

*These authors contributed equally to this work.

allocated belowground (Joseph *et al.*, 2020), supporting plant root growth and supplying rhizosphere communities with new assimilates via rhizodeposition (Epron *et al.*, 2012; Karlowsky *et al.*, 2018a; Villarino *et al.*, 2021). Extreme drought weakens the above- and belowground linkage (von Rein *et al.*, 2016; Solly *et al.*, 2023) by suppressing plant growth (Hagedorn *et al.*, 2016; Ouyang *et al.*, 2021) and/or reducing belowground C allocation (Ruehr *et al.*, 2009; Fuchslueger *et al.*, 2014; Canarini & Dijkstra, 2015; Joseph *et al.*, 2020). In turn, this may alter soil microbial community structure and functioning (Felsmann *et al.*, 2015; Karlowsky *et al.*, 2018b).

Pulse labeling experiments with carbon-13 (^{13}C) are a powerful approach to trace plant assimilates into rhizosphere communities, that is the microorganisms feeding on rhizodeposits (e.g. Streit *et al.*, 2014). The ^{13}C label can be traced into phospholipid fatty acids (PLFAs), which are essential components of the cell membrane of microbial groups in the rhizosphere. Incorporation of ^{13}C in PLFAs reflects the formation of new microbial cells and hence can serve as an indicator for the growth of rhizosphere communities (Kaiser *et al.*, 2015). In comparison, extracted microbial biomass following chloroform fumigation mainly comprises microbial cytoplasm (Hogberg *et al.*, 2010), which can also be used for other metabolic processes, for adjusting osmotic potentials (Karlowsky *et al.*, 2018b), or for storage (Mason-Jones *et al.*, 2023). In ^{13}C pulse labeling experiments in mountain grasslands, Fuchslueger *et al.* (2014) observed a reduced transfer and incorporation of assimilates into bacteria after a 2-month drought, but not into associated arbuscular mycorrhizal fungi. Growth-related lipid biomarkers showed a stronger reduction under drought than metabolic compounds, suggesting that drought shifts the metabolic turnover of rhizospheric microorganisms (Karlowsky *et al.*, 2018a). Further, rewetting of mesocosms in a mountain grassland following experimental summer drought led to rapid recovery of assimilate transfer to microbial communities and of the communities' use of the assimilates (Karlowsky *et al.*, 2018b). These results indicate high resilience of the coupling between plant and the rhizosphere communities processing new C from rhizodeposits (Ingrisch *et al.*, 2020). Less evidence is available for forests, possibly due to the methodological challenges of performing ^{13}C pulse labeling experiments with large trees (Hogberg *et al.*, 2010; Epron *et al.*, 2012), but belowground C allocation has been found to be highly sensitive to drought and subsequent rewetting (Hagedorn *et al.*, 2016; Nickel *et al.*, 2018).

Experimental approaches that have been used to explore the resistance and resilience of rhizosphere communities in forests range from microcosm experiments with saplings under controlled laboratory conditions (e.g. Solly *et al.*, 2023) to manipulation experiments in natural forest ecosystems (e.g. Wilhelm *et al.*, 2023). The magnitude of the effects of drought and rewetting often decrease with increasing scale, most likely related to the fact that more extreme conditions are generally applied in smaller-scale experiments (Gao *et al.*, 2020), to cofactors varying in natural settings, and to a higher heterogeneity in natural ecosystems combined with larger pool sizes, impeding the detection of short-term effects (Schrumpf *et al.*, 2011). Forest ecosystems with large rooting systems and undisturbed soils that have developed for centuries to millennia are particularly heterogeneous, making it challenging to translate findings from model ecosystems to natural settings.

Here, we aimed to assess the resistance and resilience of rhizosphere communities in forests to drought. We conducted our study in the context of two large ^{13}C pulse labeling experiments in two forest ecosystem types, in which we traced the transfer of tree assimilates to soil microbial communities during drought and after rewetting: (1) young beech model ecosystems, with 2.5-m-tall beech trees in open-top chambers exposed to a 2-month severe drought followed by rewetting (Hagedorn *et al.*, 2016) and (2) a 100-yr-old Scots pine forest, with 10- to 12-m-tall trees growing under natural 'moderate' summer droughts and trees growing under 15-yr experimental irrigation removing water limitation (Joseph *et al.*, 2020). In the mature forest experiment, rewetting occurred as natural precipitation after a prolonged period without rainfall. We compared the two forest ecosystem types to elucidate how drought effects observed in model ecosystems can be translated to natural forests and thus, to estimate the extent to which the responses of rhizosphere communities to drought and rewetting can be generalized. In previous assessments of these two experiments, we have shown that the transfer of new tree assimilates to soil microbial biomass and soil-respired CO_2 is reduced by drought but recovers rapidly after rewetting (Hagedorn *et al.*, 2016; Gao *et al.*, 2021). These findings indicate that C allocation from trees to the rhizosphere and the activity of the tree rhizosphere is highly responsive to temporal changes in soil moisture, even in mature forests (Joseph *et al.*, 2020).

In this study, we specifically aimed to assess how the changes in C transfer to the rhizosphere under drought and rewetting impacts the microbial communities using these rhizodeposits. We traced the ^{13}C label provided in the tree canopies of both experiments into the microbial communities, which we determined by compound-specific stable isotope analysis of PLFAs and comparing it with the ^{13}C label in microbial cytoplasm. Our hypotheses were: (1) drought would suppress the supply of recent tree assimilates to rhizosphere communities, but the communities would recover rapidly upon rewetting; (2) at the community level, fungi would be most drought resistant in processing rhizodeposits, followed by gram-positive and finally gram-negative bacteria; (3) at the cellular level, drought would suppress the use of ^{13}C-labeled rhizodeposits for new cell membrane formation more strongly than metabolic uses in the microbial cytoplasm, due to the need to maintain cells activity or to adjust osmotic potentials at the expense of growth; and (4) regarding the two forest ecosystem types, effect sizes would increase with drought severity and decrease with ecosystem complexity and thus, would be greater in the young beech model ecosystems (exposed to a severe 2-month drought) than in the mature pine forest experiencing a comparably small natural drought intensity.

Materials and Methods

General setup: model ecosystem experiment with young beech trees

In the model ecosystem study, young beech trees (*Fagus sylvatica*) were grown for 4 yr in 16 large open-top chambers (OTCs; 3 m^2,

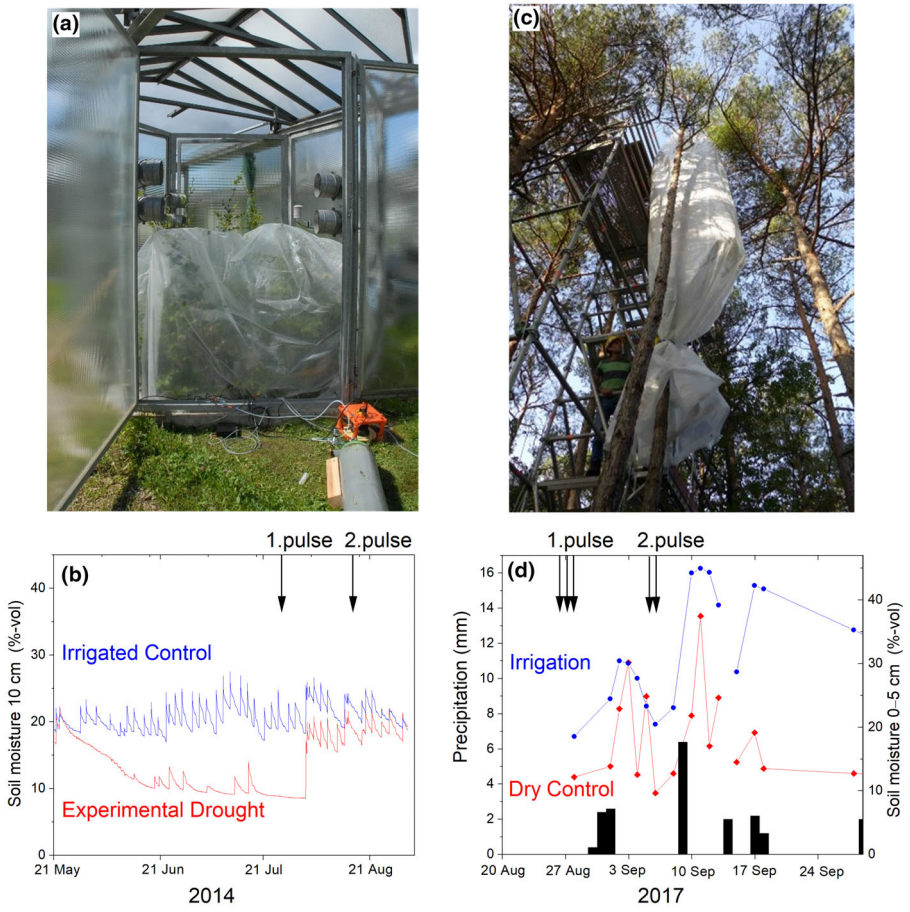

Fig. 1 The ^{13}C pulse labeling experiments in young beech model ecosystems (a, b) and in a mature pine forest (c, d). In the model ecosystems, all trees growing in one open-top chamber were covered with plastic foil (with the soil compartment additionally sealed from the air space) and together exposed to $^{13}CO_2$. In the mature forest, whole crowns of single trees were covered in transparent foil and exposed to the label. In each experiment, trees were pulse-labeled (arrows) during drought (1. pulse) and following rewetting, either after experimental rewetting or after natural rainfall (2. pulse). The relative soil moisture at 10 cm depth in the model ecosystems is given in (b), while values at 0–5 cm depth in the mature pine forest are given in (d). The black bars in (d) indicate natural precipitation events. There were three replicates in the dry period and four and two replicates after rewetting in the young beech model ecosystems and in the mature pine forest, respectively.

height 3.5 m; Fig. 1, Supporting Information Fig. S1). Each OTC consisted of two 1.5-m deep lysimeters. One of them was used for the pulse labeling and had been filled with a sandy acidic forest soil with low soil organic C content (0.48%; Table 1). Twenty-four beech trees had been planted in each lysimeter. The OTCs had automated irrigation systems and sliding roofs that closed automatically during rainfall. During the growing season, the OTCs were irrigated every 2 or 3 d with 67 l m^{-2} of artificial rainwater with a chemical composition similar to natural rainfall (for details see Hagedorn *et al.*, 2016). Four years after their planting, when the trees had reached a height of 2.5 m, half (eight) of the OTCs were exposed to an experimental drought from 22 May until 1 August, which reduced the water supply by 78% compared with controls ($n = 8$ OTCs). Thereafter, these OTCs were rewetted with 200 l m^{-2} for 1 d, followed by the same irrigation regime as in the continuously irrigated control OTCs.

Table 1 Physical and chemical characteristics in the two forest ecosystems used for the pulse labeling study.

Experiment	Young beech model ecosystems	Mature pine forest
pH (0.01 M $CaCl_2$)	4	6.5 ± 0.02
Soil texture		
Sand (%)	85	51 ± 1.4
Silt (%)	8	38 ± 0.7
Clay (%)	5	11 ± 0.7
Stone content (g kg^{-1})	0	200 ± 50
Soil organic carbon (g C kg^{-1})	4.8	75 ± 8
Total nitrogen (g N kg^{-1})	0.3	3.2 ± 0.3
C : N	16	23.6 ± 0.4
CEC (mmol kg^{-1})	24.1	303 ± 21

Mean and SEs of four dry control plots in the mature pine forest. In the young beech model ecosystems, thoroughly mixed soil had been filled into the lysimeters of the open-top chambers. C : N, ratio of soil total carbon to total nitrogen; CEC, cation exchange capacity.

Long-term irrigation experiment in a mature dry pine forest

The mature pine forest is a naturally regenerated forest, dominated by *c.* 120-yr-old Scots pine (*Pinus sylvestris*; 10–12 m tall), located in the dry inner-Alpine valley of the Rhone river in southwestern Switzerland, close to the dry edge of the natural distribution of the species. The research site (46°18′N, 7°37′E, 615 m a.s.l.; Bose *et al.*, 2022) experiences a moderately continental climate with a mean annual temperature and precipitation of 10.6°C and *c.* 575 mm, respectively. Soils are shallow Para-rendzina with a mull- and Xeromoder-type organic layer developed on an alluvial fan (Guidi *et al.*, 2022). The experimental

site of 1.2 ha was randomly divided into eight plots of 25 × 40 m (1000 m^2) each, separated by a 5-m-wide buffer area (Guidi *et al.*, 2022; Fig. S2). Irrigated plots were watered from an adjacent hydrologically disconnected channel of the Rhone river. Four randomly selected plots (further termed 'irrigated') were irrigated by sprinklers with 5 mm of water per day on rainless nights during the vegetation period (from May to October), doubling rainfall compared with the other four plots with naturally dry conditions (further termed 'dry control').

^{13}C pulse labeling

In both forest ecosystem types, ^{13}C pulse labeling was applied in two campaigns; the first one at the end of a drought period and the second one *c.* 2 wk after rewetting. In the young beech model ecosystems, ^{13}C pulse labeling was conducted in six randomly selected OTCs during drought (three experiencing drought and three receiving irrigation) in mid July 2014 and in eight OTCs 2 wk after rewetting (four experiencing rewetting after drought and four receiving continuous irrigation) beginning of August. To avoid the influence of previous ^{13}C pulse labeling on the results, different OTCs were used for the two different times of the pulse labeling experiments (Fig. S1). All 24 trees in each OTC were placed in custom-made 3-m-tall tents of transparent plastic foil. The tents were flushed with CO_2-free air, and the systems were then enriched with ^{13}C-labeled $^{13}CO_2$ (50 : 50 of $^{12}CO_2$ and $^{13}CO_2$ with 99% atom excess) for 2 h, keeping a constant CO_2 concentration of 1500 ppm (for further details see Hagedorn *et al.*, 2016).

In the mature pine forest, 12-m-tall scaffolds were installed near tree canopies ($n = 3$ per treatment) to allow canopy access. Here, we used the scaffolds to facilitate the whole-tree ^{13}C pulse labeling of 10 trees (five trees in three irrigated plots and five trees in three dry control plots; Figs 1, S2). For the pulse labeling, whole-tree canopies were wrapped with transparent plastic bags, and highly enriched $^{13}CO_2$ (99% atom excess) was then added for 3 h. During the pulse labeling, concentrations of $^{13}CO_2$ in the plastic bags were maintained at *c.* 1500 ppm (for details see Joseph *et al.*, 2020). The first pulse labeling campaign was performed with three pairs of trees (one drought-exposed and one irrigated) at the end of August when the dry control plots experienced a moderate drought ($n = 3$) while the other plots were continuously irrigated ($n = 3$). The second labeling campaign was conducted 2 wk after a precipitation event at the beginning of September 2017, using two pairs of trees ($n = 2$ experiencing rewetting after drought; $n = 2$ receiving continuous irrigation).

CO_2 flux rates from soil, and soil sampling

In both experiments, soil CO_2 flux and $\delta^{13}C$-CO_2 were measured from polyvinyl chloride (PVC) collars (10.5 cm in diameter) inserted into soils to 3 cm depth. Two collars per OTC were used in the young beech ecosystems, while in the pine forest collars placed at 0.5 and 1 m distance from each of the labeled trees were used for sampling along three gradients ($n = 6$ per tree). Soil CO_2 efflux was measured with a Li-8100 soil CO_2 flux system (LI-COR Inc., Lincoln, NE, USA). The $\delta^{13}C$ value of soil-respired CO_2 was determined using the closed-chamber method, that is by closing each collar for 15–30 min with a PVC lid, depending on efflux rates. Gas samples were retrieved and stored for no more than 1 wk in 12 ml glass vials (Exetainer®; Labco, Lampeter, UK) before isotopic measurements. Two additional samples were taken from ambient air near each tree. Gas samples were analyzed with a Gas Bench II linked to a Delta V Plus mass spectrometer (Thermo Finnigan MAT, Bremen, Germany).

In the beech model ecosystems, soils (5–10 cm depth) were repeatedly sampled at three randomly selected locations per lysimeter using a 2-cm-diameter soil auger. The soil samples were thoroughly mixed and passed through a 4-mm sieve while removing fine roots and stones. The samples were then immediately transported to the laboratory, where they were frozen at −20°C to prevent further processing of ^{13}C-labeled components until analysis. In the mature pine forest, soil samples were taken at 0–2, 2–5, and 5–10 cm depth using the same 2-cm auger. To obtain representative samples, 12 individual samples taken within 1 m distance from each tree trunk were combined. These samples were evenly distributed among the three cardinal directions. Following collection, these soil samples were thoroughly mixed and passed through a 4-mm sieve. All soil samples were then immediately frozen and stored in a field freezer until they were all transported to the laboratory. In both experiments, soil gravimetric water content was assessed by drying the soil at 105°C for 24 h. Values were converted to volumetric water contents using measured soil bulk density. Sampling dates were adjusted to match the appearance of the ^{13}C signal in rhizosphere respiration based on continuous monitoring with laser systems (LGR-CCIA 36-d; LosGatos Research Ltd, San Francisco, CA, USA; see Hagedorn *et al.*, 2016; Joseph *et al.*, 2020).

Soil microbial biomass carbon

The chloroform fumigation extraction (CFE) method was used to determine soil microbial biomass carbon (MBC; Joergensen, 1996). This involved extracting unfumigated and fumigated soils with 0.5 M K_2SO_4 solution in a ratio of 1 : 4. While MBC contents were taken from published work (Hagedorn *et al.*, 2016; Gao *et al.*, 2021), the $\delta^{13}C$ values in MBC were determined by analyzing the $\delta^{13}C$ values in the extracts of the unfumigated and fumigated soils in this study. The extractable organic C was first oxidized with $K_2S_2O_8$ and H_3PO_4 (Lang *et al.*, 2012), and $\delta^{13}C$ values and concentrations of released CO_2 were then measured, as described above for soil-respired CO_2. Finally, MBC contents were estimated by calculating the differences in extractable organic C between fumigated and unfumigated soils and dividing these values by 0.45 (Joergensen, 1996).

PLFA analysis

Phospholipid fatty acids (PLFAs) were analyzed with a modified Bligh-Dyer method (Steger *et al.*, 2011). Briefly, 2 g of freeze-dried soil was extracted using a mixture of phosphate buffer, chloroform, and methanol (0.8 : 1 : 2 v/v/v). Phospholipids were then separated from total lipids using a silicic acid column

(BondElut LRC-Si; Agilent Technologies Inc., Santa Clara, CA, USA). Methyl nonadecanoate (19 : 0; Sigma-Aldrich, St Louis, MO, USA) was added to the samples as an internal standard for quantifying PLFAs. PLFAs concentrations were identified by gas chromatography (Agilent 7890; Agilent Technologies) with an Ultra-2 column. The $\delta^{13}C$ values of individual PLFAs were determined using a gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS: HP5890 GC; Agilent Technologies) coupled to a Delta V Plus mass spectrometer (DELTA Plus XP; Thermo Finnigan, Bremen, Germany).

The PLFAs 18:1ω9 and 18:2ω6,9 are considered markers for fungi (Kaiser *et al.*, 2010). As 18:2ω6,9 is most closely associated with the tree rhizosphere (Streit *et al.*, 2014), its ^{13}C label can be regarded as an indicator for ectomycorrhizal fungi (Hogberg *et al.*, 2010). The PLFAs 16:1ω7, 18:1ω7, cy17:0, and cy19:0 are used as markers for gram-negative bacteria (Zelles, 1999); i15:0, a15:0, i16:0, i17:0, and a17:0 for gram-positive bacteria (Zelles, 1997); 10Me16:0 and 10Me18:0 for actinobacteria (Zelles, 1999); and 16:1w5 for arbuscular mycorrhizal fungi (AMF) (Joergensen, 2022).

Calculations and statistical analyses

Mass balance equations were used to estimate $\delta^{13}C$ values of soil-respired CO_2 considering chamber and ambient air (Streit *et al.*, 2014), as well as in MBC based on extracts of fumigated and unfumigated soils (e.g. Gao *et al.*, 2021; Notes S1). For the ^{13}C enrichment resulting from the ^{13}C input of rhizodeposits into soil CO_2 efflux, MBC or PLFAs, $\Delta\delta^{13}C$ was calculated as the difference in $\delta^{13}C$ values of pulse-labeled and nonlabeled trees. The total ^{13}C mass (^{13}C excess) for CO_2, MBC or PLFA was calculated according to Gao *et al.* (2021), that is by multiplying pool sizes by the *atom fraction* based on the ^{13}C label strength (Notes S1). The uptake of ^{13}C by trees was estimated by sampling foliage after pulse labeling and converting the ^{13}C label of these leaves to ^{13}C excess by measuring foliage biomass after the experiment for the beech model ecosystems (Hagedorn *et al.*, 2016) and by applying an allometric tree function for the pine forest (Joseph *et al.*, 2020). In addition, the response ratio, that is the logarithm of the ratio of the values of drought-exposed trees to the values of irrigated trees ($\log_e$(drought/irrigated)), was used to explore the magnitude of the drought effect on variables.

Data were analyzed by fitting linear mixed effects models with maximum likelihood, using the 'lme' function in the NLME package (v.3.1-162) in R software v.3.5.3 (R Development Core Team, 2018). In the models, experiment (mature pine forest vs young beech model ecosystems), timing (labeling campaigns before rewetting vs after rewetting), and treatment (irrigated vs drought) were included as fixed effects, and individual trees and the tree pairs used in the pair-wise pulse labeling were included as random effects. In models that considered data from all dates, an autocorrelation structure ('corAR1' function in the NLME package) was included to account for repeated measurements with a first-order autoregressive covariate structure. *T*-tests were carried out to test whether there were statistically significant differences in $^{13}CO_2$, ^{13}C-MBC, and ^{13}C-PLFA between the treatments overall or at single time points. In addition, principal component analysis (PCA) and partial redundancy analysis (RDA) were performed to evaluate changes in soil microbial community composition under the different treatments in both experiments. All statistical analyses were performed and all figures were created using R software v.3.5.3. Reported values are mean ± SE.

Results

Drought and rewetting patterns

Drought significantly reduced soil water content in both experiments. In the young beech model ecosystems, soil water content decreased to 8.5%-vol at 10 cm depth during the 2-month experimental drought (Fig. 1). The continuously irrigated soils maintained a constant soil water content of 21%-vol. Rewetting immediately increased the soil water content of dry soils to almost the same level as in the irrigated OTCs. In the mature pine forest, the natural drought was more moderate, with soil water content reaching a minimum of 13%-vol. There was a precipitation event between the two pulse-labeling campaigns, which increased soil water content to 30%-vol in the uppermost 5 cm, with values briefly reaching levels similar to those in the continuously irrigated soils before the rainfall (Fig. 1). The rewetting effect was confined to the upper 5 cm of soil (see Joseph *et al.*, 2020).

Microbial biomass and community structure

Microbial biomass and the concentrations of total PLFAs and specific PLFA biomarkers were substantially smaller in soils of young beech model ecosystems (SOC content 4.8 g C kg^{-1}) than in the mature pine forest (SOC content 75 g C kg^{-1}; Tables 1, 2). Conversely, the ratio of gram-positive to gram-negative bacteria was significantly higher in the beech model ecosystems than in the mature pine forest ($P < 0.0001$, Table 2). There were no significant differences between the two forest types in the ratio of fungi to bacteria ($P = 0.922$, Table 2). Microbial biomass and total PLFAs remained unaffected by the water regime (Table 2). Similarly, the ratios of fungi to bacteria and of gram-positive to gram-negative bacteria showed no overall drought effect (Table 2). However, when the experiments were analyzed separately, the ratio of gram-positive to gram-negative bacteria was significantly higher in dry than in irrigated soils in the pine forest ($P < 0.001$) but remained unaffected by drought in the beech model ecosystems ($P = 0.22$; Table 2). The PCA of all PLFAs components confirmed this result, revealing distinctly different communities at the group level under natural drought and long-term irrigation in the mature pine forest, while microbial communities remained unaffected in the beech model ecosystems (Fig. S3).

^{13}C tracing

Under constantly moist conditions, the ^{13}C pulse added to tree crowns appeared in soil-respired CO_2 and in CFE-microbial

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Table 2 Concentrations of the phospholipid fatty acids (PLFAs) of microbial groups and statistical significance of experiment and water regime.

Experiment	Timing	Treatment	Gram-positive bacteria nmol g^{-1}	Gram-negative bacteria nmol g^{-1}	Bacteria nmol g^{-1}	Actinomycete nmol g^{-1}	Fungi nmol g^{-1}	AM fungi nmol g^{-1}	Total PLFAs nmol g^{-1}	$G^+ : G^-$	F : B	Microbial biomass mg kg^{-1}
Young beech model ecosystems	Dry period	Irrigated	18.4 ± 1.6	16.3 ± 1.2	34.7 ± 2.8	5.2 ± 0.6	10.7 ± 1.4	5.2 ± 0.6	55.8 ± 4.1	1.2 ± 0.04	0.31 ± 0.03	83.9 ± 2.2
		Drought	21.3 ± 2.1	19.2 ± 2.0	40.5 ± 4.1	4.9 ± 0.9	11.2 ± 1.2	5.4 ± 0.5	62.0 ± 4.8	1.1 ± 0.01	0.28 ± 0.04	85.0 ± 3.3
	Rewetted	Irrigated	18.8 ± 1.1	15.5 ± 1.8	34.3 ± 2.7	4.7 ± 0.3	13.1 ± 0.7	4.9 ± 0.3	57.0 ± 3.4	1.3 ± 0.12	0.38 ± 0.01	115 ± 8.1
		Rewetted	18.6 ± 1.2	16.5 ± 0.7	35.1 ± 1.9	5.4 ± 0.4	11.6 ± 1.4	3.3 ± 0.7	55.4 ± 2.6	1.1 ± 0.02	0.33 ± 0.04	113 ± 2.8
Mature pine forest	Dry period	Irrigated	26.9 ± 1.6	49.6 ± 1.9	76.5 ± 3.5	9.3 ± 0.4	22.8 ± 1.5	4.9 ± 0.2	114 ± 5.4	0.54 ± 0.01	0.30 ± 0.01	2187 ± 174
		Drought	21.3 ± 0.9	36.3 ± 0.8	57.6 ± 1.4	6.5 ± 0.2	20.8 ± 0.9	3.9 ± 0.2	88.7 ± 2.4	0.58 ± 0.02	0.35 ± 0.01	1661 ± 149
	Rewetted	Irrigated	17.8 ± 0.1	38.9 ± 0.7	56.7 ± 0.8	5.6 ± 0.1	19.2 ± 1.3	4.4 ± 0.02	85.9 ± 0.4	0.46 ± 0.01	0.34 ± 0.03	1270 ± 4.8
		Rewetted	23.3 ± 2.1	35.1 ± 3.0	58.5 ± 5.1	7.4 ± 1.0	19.4 ± 0.6	3.6 ± 0.12	88.8 ± 6.7	0.66 ± 0.01	0.33 ± 0.02	1553 ± 233
Statistical significance	Experiment		**0.0300**	**0.0001**	**0.0003**	**0.0040**	**0.0008**	0.0710	**0.0002**	**<0.0001**	0.9220	**<0.0001**
	Treatment		0.768	0.737	0.730	0.860	0.440	0.057	0.405	0.367	0.555	0.477
	Timing		0.092	0.108	0.096	0.330	0.770	0.071	0.113	0.604	0.079	0.316
	Experiment × Treatment		0.371	**0.032**	0.057	0.277	0.976	0.340	0.082	**0.022**	0.190	0.398
	Experiment × Timing		0.328	0.704	0.473	0.195	0.126	0.357	0.249	0.345	0.287	**0.004**
	Treatment × Timing		0.362	0.649	0.614	**0.042**	0.649	0.292	0.688	0.385	0.365	0.134
	Experiment ×Treatment× Timing		**0.033**	0.213	0.090	0.177	0.331	0.159	0.063	0.057	0.718	0.068
Young beech model ecosystems	Treatment		0.499	0.236	0.331	0.620	0.624	0.110	0.649	0.220	0.242	0.989
	Timing		0.497	0.291	0.365	0.903	0.276	0.067	0.512	0.382	0.092	**0.0001**
	Treatment × Timing		0.331	0.702	0.465	0.305	0.391	0.107	0.336	0.446	0.670	0.847
Mature pine forest	Treatment		0.578	**0.002**	**0.013**	0.101	0.401	**0.003**	**0.022**	**0.001**	0.131	0.375
	Timing		**0.028**	**0.018**	**0.022**	**0.019**	0.084	0.152	**0.022**	0.397	0.541	**0.020**
	Treatment × Timing		**0.004**	0.053	**0.016**	**0.002**	0.406	0.804	**0.022**	**0.002**	0.080	0.051

Soils were analyzed at two time points (included in linear mixed effects models as the fixed effect 'timing'), during drought and 2 wk after rewetting. Hence, drought–rewetting effects were tested in the models with interaction term by 'treatment × timing' where the treatments were continuous irrigation vs drought conditions during a dry period and after rewetting. $G^+ : G^-$: ratio of gram-positive bacteria to gram-negative bacteria; F : B: ratio of fungi to bacteria; AM fungi: arbuscular mycorrhizal fungi. Values are means ± SEs. There were three replicates in the dry period in both experiments and four and two replicates after rewetting in the young beech model ecosystems and the mature pine forest, respectively. Bold values represent statistically significant differences ($P < 0.05$).

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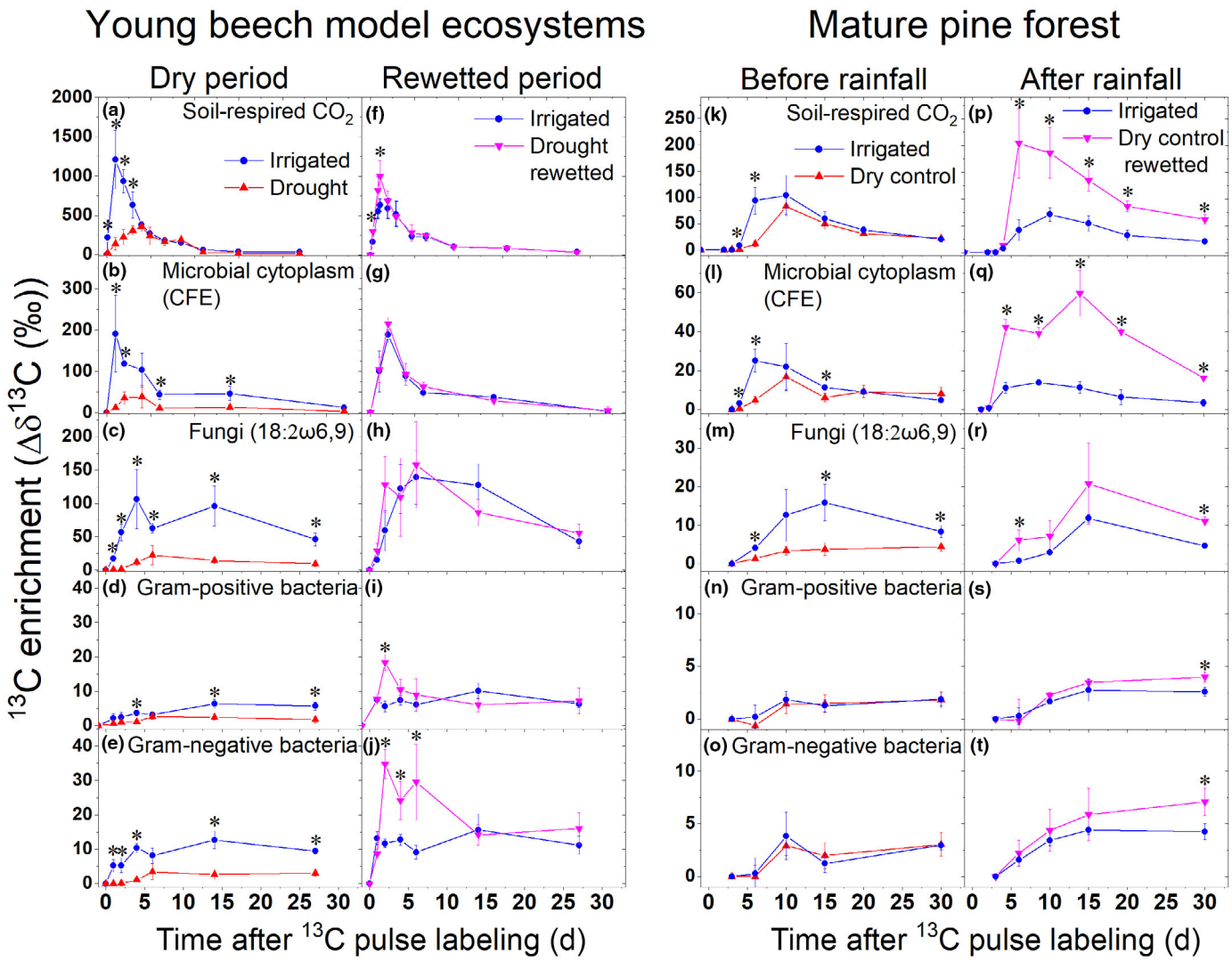

Fig. 2 The ^{13}C signal derived from ^{13}C pulse labeling ($\Delta\delta^{13}C = \delta^{13}C_{pulse} - \delta^{13}C_{ambient}$) under drought and 2 wk after rewetting in young beech model ecosystems (0–10 cm depth; a–j) and in a mature pine forest (0–5 cm depth; k–t). Means and SEs are shown of three replicates in the dry period and of four and two replicates after rewetting in the young beech model ecosystems and mature pine forest, respectively. The data on soil-respired CO_2 and CFE-microbial biomass are taken from Hagedorn *et al*. (2016) and Gao *et al*. (2021). Note that the scale of the ^{13}C enrichment is different for the two ecosystem types. An asterisk indicates a significant difference between the drought or drought–rewetted and the irrigated treatments at a given time point based on *t*-tests ($P < 0.05$). CFE, chloroform fumigation extraction.

biomass *c*. 1 d after labeling in the beech model ecosystems and 4 d after labeling in the mature pine forest (Fig. 2). The $\Delta\delta^{13}C$ values reflecting the ^{13}C enrichment from the pulse labeling was greater in the beech model ecosystems than in the mature pine forest. The appearance of the ^{13}C label was significantly delayed in PLFAs compared with in microbial biomass, peaking 15 d after the labeling in the fungal biomarker 18:2ω6,9 in the mature pine forest. In gram-positive and gram-negative bacteria, the label remained constant until day 30 (Fig. 2). In both forest types, the $\Delta\delta^{13}C$ values were much greater in CFE-microbial biomass than in PLFAs (Fig. 2; Table S1). Within PLFAs, $\Delta\delta^{13}C$ values showed different patterns among microbial groups, with the strongest signal in the fungal biomarker 18:2ω6,9 (Fig. 2). Gram-positive and gram-negative bacteria had similar ^{13}C enrichments. Actinobacteria did not incorporate a significant ^{13}C label, with $\Delta\delta^{13}C$ values not exceeding SDs of unlabeled trees.

Drought conditions decreased ^{13}C uptake by trees by 84% in the beech model ecosystems but only by 17% in the mature pine forest ($P_{Drought} < 0.05$; Table 3; Hagedorn *et al*., 2016; Joseph *et al*., 2020). Under drought, the transfer of ^{13}C-labeled assimilates into the rhizosphere was delayed compared with under irrigated conditions, and $\Delta\delta^{13}C$ values were lower in soil-respired CO_2 and CFE-microbial biomass ($P_{Drought} < 0.05$; Figs 2, 3; Table 3). The response ratios for peaks of ^{13}C enrichment were higher in young beech ecosystems exposed to strong experimental drought than in the mature pine forest experiencing moderate natural drought ($P_{Experiment \times Drought} < 0.05$; Fig. 3; Table S1). Overall, drought suppressed ^{13}C enrichment in PLFAs, but

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Table 3 ^{13}C excess in tree biomass, soil-respired CO_2, chloroform fumigation extraction (CFE)-microbial biomass, and in microbial groups assessed by phospholipid fatty acids (PLFAs) and statistical significance of experiment and water regime.

Experiment	Timing	Treatment	Tree uptake mg ^{13}C m^{-2}	Respired CO_2 (30 d) mg ^{13}C m^{-2} 30 d^{-1}	Microbial biomass mg ^{13}C kg^{-1}	PLFA 18:2w6,9 nmol ^{13}C kg^{-1}	Gram-positive bacteria nmol ^{13}C kg^{-1}	Gram-negative bacteria nmol ^{13}C kg^{-1}
Young beech model ecosystems	Dry period	Irrigated	1163 ± 117	218 ± 34	0.06 ± 0.02	7.9 ± 4.4	1.8 ± 0.11	2.8 ± 0.24
		Drought	186 ± 92	52 ± 13	0.01 ± 0.004	1.8 ± 1.2	0.79 ± 0.20	1.5 ± 0.38
	Rewetted	Irrigated	822 ± 189	163 ± 58	0.06 ± 0.01	15.5 ± 4.3	2.5 ± 0.57	4.8 ± 1.2
		Rewetted	906 ± 203	270 ± 58	0.11 ± 0.02	17.1 ± 9.0	4.6 ± 0.73	9.1 ± 2.6
Mature pine forest	Dry period	Irrigated	9913 ± 946	1844 ± 271	0.55 ± 0.28	2.5 ± 0.4	0.93 ± 0.04	3.0 ± 0.17
		Drought	8200 ± 977	694 ± 95	0.30 ± 0.09	0.7 ± 0.1	0.60 ± 0.15	1.6 ± 0.33
	Rewetted	Irrigated	13 350 ± 58	2002 ± 136	0.22 ± 0.07	1.2 ± 0.1	0.35 ± 0.01	1.5 ± 0.05
		Rewetted	11 777 ± 776	1499 ± 149	1.18 ± 0.43	2.2 ± 1.1	0.87 ± 0.10	2.0 ± 0.15
Statistical significance		Experiment	**0.0001**	**0.0002**	**0.0008**	**0.007**	**0.002**	**0.020**
		Treatment	0.061	0.051	0.884	0.140	0.857	0.594
		Timing	**0.021**	**0.007**	**0.040**	**0.016**	**0.008**	**0.004**
		Experiment × Treatment	0.120	0.281	0.200	0.677	0.850	0.369
		Experiment × Timing	0.470	0.653	0.153	0.102	**0.014**	**0.017**
		Treatment × Timing	**0.035**	**0.011**	**0.011**	0.093	**0.008**	**0.023**
		Experiment ×Treatment× Timing	0.052	0.110	0.937	0.823	0.762	0.597
	Dry period	Experiment	**0.0006**	**0.0003**	**0.003**	0.139	0.084	0.635
		Drought	**0.033**	**0.003**	0.066	**0.028**	**0.025**	**0.025**
		Experiment × Drought	**0.034**	0.275	0.263	0.774	0.356	0.905
	Rewetted period	Experiment	0.057	0.081	0.082	0.092	0.053	0.087
		Rewetted	0.947	0.454	0.152	0.959	0.305	0.980
		Experiment × Rewetted	0.731	0.357	0.261	0.748	0.427	0.283
	Effect size		0.99	1.20	3.58	1.49	2.17	1.62

Peak ^{13}C excess values were used for microbial biomass and PLFAs. Soils were analyzed in two time points (included in linear mixed models as the fixed effect 'timing'), during drought and 2 wk after rewetting. Hence, drought effects were tested with the fixed effect 'drought' in the dry period and drying–rewetting effects were tested with the interaction term 'drought × timing'. 'Treatment' includes the drought treatment during the dry period and after rewetting. Values are means ± SEs of three replicates in the dry period and of four and two replicates after rewetting in the young beech model ecosystems and mature pine forest, respectively. Bold values indicate statistically significant differences ($P < 0.05$). The data on soil-respired CO_2 and CFE-microbial biomass are taken from Hagedorn *et al*. (2016) and Gao *et al*. (2021).

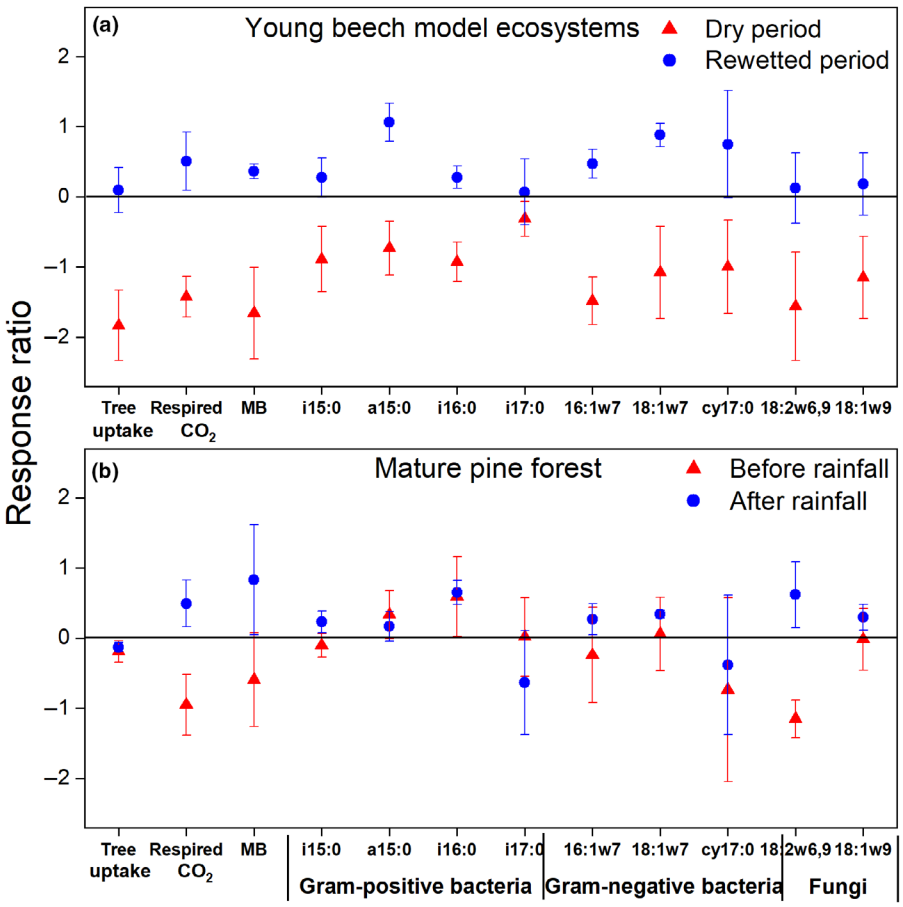

Fig. 3 Response ratio of ^{13}C signals derived from ^{13}C pulse labeling under drought and 2 wk after rewetting in young beech model ecosystems (0–10 cm, a) and in a mature pine forest (0–5 cm, b). For tree uptake and soil respiration, the response ratio was estimated from ^{13}C excess, while peak $\Delta\delta^{13}C$ values were used for microbial biomass (MB) and phospholipid fatty acids. Note that a response ratio of 0.69 corresponds to a doubling. Statistical effects of ^{13}C excess and peak $\Delta\delta^{13}C$ values can be found in Table 3 and Supporting Information Table S1, respectively. Means and SEs are shown of three replicates in the dry period and of four and two replicates after rewetting in the young beech model ecosystems and mature pine forest, respectively. The data on soil-respired CO_2 and chloroform fumigation extraction (CFE)-MB are taken from Hagedorn *et al*. (2016) and Gao *et al*. (2021).

responses varied strongly among individual PLFAs and microbial groups (Figs 2, 3). The fungal PLFA 18:2ω6,9 showed the strongest drought-induced reduction in $\Delta\delta^{13}C$, by 85% averaged across the two forest types ($P_{Drought} < 0.001$). Gram-positive bacteria were the least affected by drought, decreasing in $\Delta\delta^{13}C$ by 65% and 23% in the beech model ecosystems and pine forest, respectively (Table S1).

Two weeks after rewetting, ^{13}C uptake by previously drought-affected trees was the same as by continuously irrigated trees (Table 3). In rewetted soils in both experiments, the ^{13}C enrichment of soil-respired CO_2, CFE-microbial biomass, and PLFAs of fungi and gram-negative bacteria strongly increased compared with during the drought period (Figs 2, 3; $P_{Drought \times Timing} < 0.05$; Table S1). The ^{13}C incorporation into the microbial communities of the rewetted soils even surpassed that in the constantly moist control soils, reflected in positive response ratios for almost all PLFAs (except i17:0; Fig. 3). However, when tested solely for the rewetted period, this effect was only significant for gram-negative and gram-positive bacteria in the beech model ecosystems. During this period, the response ratios did not differ significantly from zero for soil-respired CO_2, CFE-microbial biomass, or fungal PLFAs, although their peak $\Delta\delta^{13}C$ values were 88–130% higher in previously drought-exposed, rewetted soils than in continuously moist soils.

The ^{13}C excess in PLFAs showed the same pattern, with smaller values under drought than under irrigation during the dry period ($P_{Drought} < 0.05$; Table 3), but higher values upon rewetting ($P_{Drought \times Timing} < 0.05$). Although ^{13}C excess was 83%, 148%, and 33% higher in fungi, gram-negative, and gram-positive bacteria in rewetted than in continuously moist soils, these effects were statistically nonsignificant when tested for the rewetted period only (Fig. 4; Table 3).

Ratios of $\Delta\delta^{13}C$ of total PLFAs to CFE-microbial biomass were significantly affected by the water regime in both forest types ($P_{Drought \times Timing} < 0.05$), with smaller values under drought (Fig. 5; Table 2). After rewetting, the ratio increased strongly in the beech model ecosystems. A rewetting effect did not occur in the pine forest with a milder drought. PLFAs suppression under drought was most pronounced for the fungal biomarker 18:2ω6,9 ($P_{Drought} < 0.01$ in both experiments; Table S2). For gram-positive and gram-negative bacteria, rewetting increased the ratio in the young beech model ecosystems ($P_{Drought} < 0.05$) but decreased it in the mature pine forest ($P_{Drought} < 0.05$; Table S2).

Discussion

Our ^{13}C pulse labeling experiments reveal a tight coupling of above- and belowground communities in forest ecosystems. Carbon assimilated in the tree canopy was transferred to microbial communities colonizing the rhizosphere within 1 and 4 d

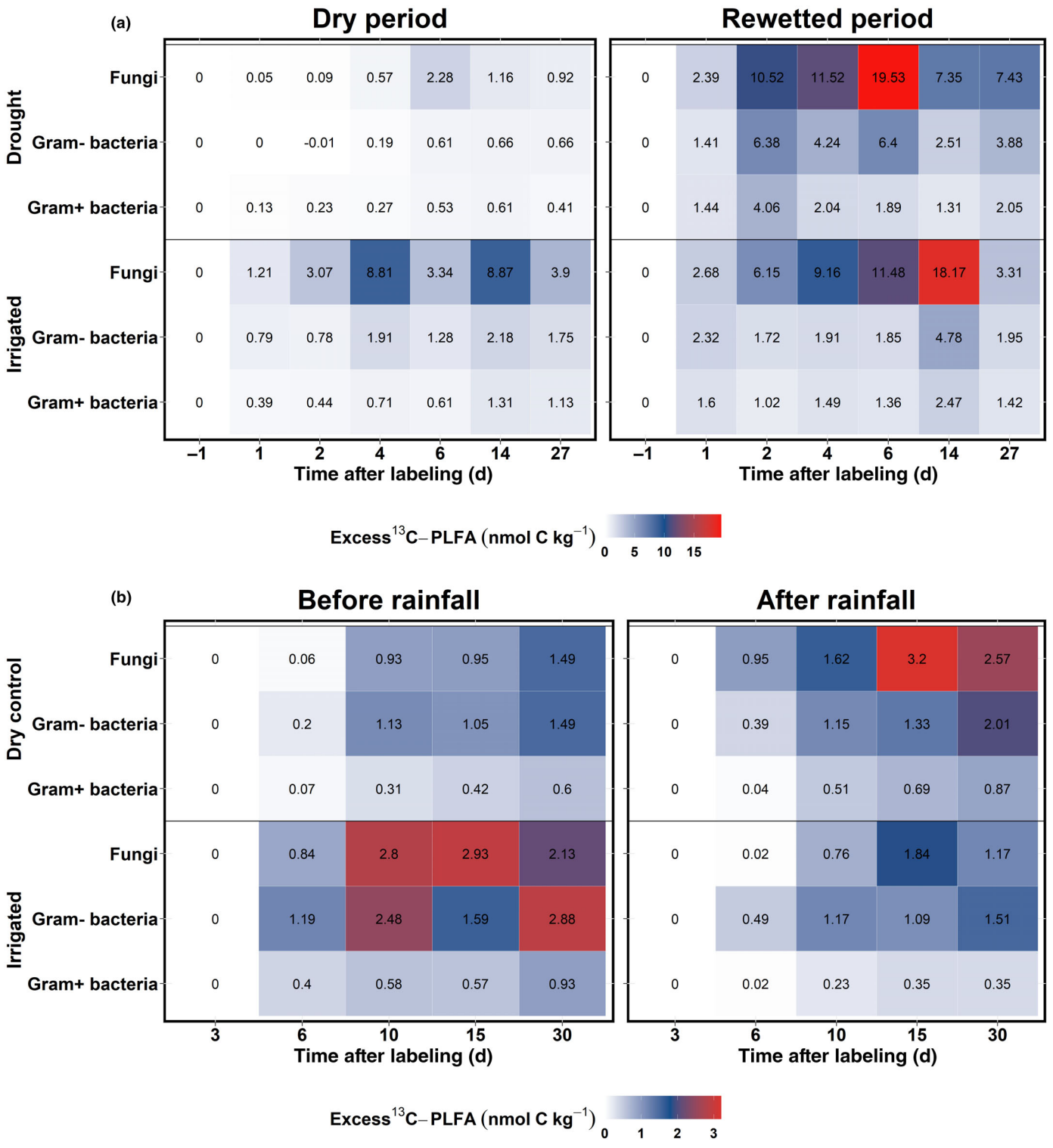

Fig. 4 Temporal dynamics of ^{13}C excess in phospholipid fatty acids in microbial communities under drought and rewetting in the young beech model ecosystems (a) and in the mature pine forest (b). Means are shown of three replicates in the dry period and of four and two replicates after rewetting in the young beech model ecosystems and mature pine forest, respectively.

of labeling in the young beech forest model ecosystems and mature Scots pine forest, respectively. In both forest types, incorporation of the ^{13}C label by the rhizosphere communities was strongly suppressed by drought but recovered when the label was applied 2 wk following rewetting. This implies that C allocation to the belowground and the use of rhizodeposits by the microbial community are highly resilient in the face of drought.

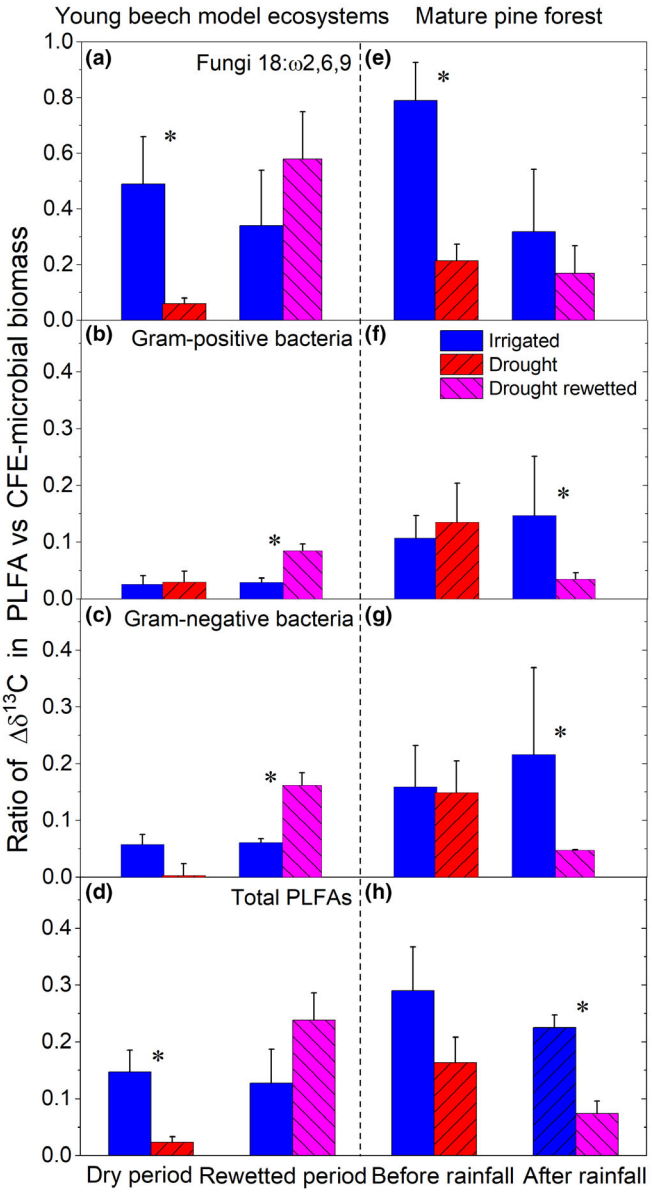

Fig. 5 Ratios of $\Delta\delta^{13}C$ in phospholipid fatty acids (PLFAs) vs in chloroform fumigation extraction (CFE)-microbial biomass at the peak of the ^{13}C signal, 2 and 10 d after pulse labeling in the young beech model ecosystems (a–d) and in the mature pine forest (e–h), respectively. The incorporation of new assimilates into PLFAs, that is major components of cell membrane, signifies the formation of new cells. Meanwhile, ^{13}C inputs into CFE-microbial biomass, primarily composed of microbial cytoplasm, encompass carbon used for osmoregulation, storage, and metabolic processes. Means and SEs are shown of three replicates in the dry period and of four and two replicates after rewetting in the young beech model ecosystems and mature pine forest, respectively. An asterisk indicates a significant difference between the drought or drought–rewetted and the irrigated treatments based on *t*-tests ($P < 0.05$).

Drought reduces ^{13}C incorporation by rhizosphere communities

The reduced rhizodeposition and slower C processing by rhizosphere communities in the two forest types under drought agrees with findings from ^{13}C pulse labeling experiments in grasslands, where microbial communities received and processed assimilates within a few hours (Fuchslueger *et al.*, 2016; Karlowsky *et al.*, 2018b). However, as opposed to in grassland experiments, where allocation of assimilates to fungal symbionts, in particular, remained unaffected by drought (Bahn *et al.*, 2013; Fuchslueger *et al.*, 2016), we observed the strongest decrease under drought for ^{13}C incorporation into fungal 18:2ω6,9 (Fig. 3), serving as a biomarker for ectomycorrhizal (ECM) fungi (Hogberg *et al.*, 2010). One reason for the divergent drought responses of fungal symbionts in grasslands and forests could be that arbuscular (AM) fungi associated with graminoids are less sensitive to drought than ECM fungi associated with trees (Gehring *et al.*, 2017). However, comparative studies between mycorrhizal types and species are rare. Unfortunately, tracing ^{13}C into PLFAs can only elucidate microbial substrate use at the group level. The ^{13}C label strength provided by ^{13}C enrichment experiments in forests is too small for stable isotope probing (SIP), which would make it possible to determine ^{13}C incorporation also at the family or genus level (Dumont & Murrell, 2005). In the same long-term irrigation experiment in the mature pine forest as in our study, 454-pyrosequencing of ribosomal marker genes by Hartmann *et al.* (2017) revealed bidirectional drought effects on typical ECM Agaricomycetes, which responded either negatively (e.g. Inocybe, Rhizopogon) or positively (e.g. Tricholoma, Craterellus) relative to under irrigated conditions. Nonetheless, our ^{13}C pulse labeling experiments in both forest types reveal that drought reduces the assimilate supply of fungal symbionts. As mycorrhizal fungi facilitate water and nutrient uptake by trees (Puschel *et al.*, 2020), this in turn may weaken the overall resistance of forests to drought.

The suppressed incorporation of the ^{13}C label by rhizosphere communities under drought could be driven by water limitation of microbial activity (Schimel *et al.*, 2007) and/or by less assimilate transfer from trees (Prescott *et al.*, 2020), resulting from reduced ^{13}C uptake by trees, slower formation of sugars in foliage (Ruehr *et al.*, 2009), and slower assimilate transport via phloem tissue (Gao *et al.*, 2021; Werner *et al.*, 2021; Hikino *et al.*, 2022). Our findings support both mechanisms. In the beech model ecosystems, ^{13}C uptake by trees was strongly reduced under severe drought (−84%; Table 3), with drought also decreasing the amount of assimilates available for belowground allocation. This result strongly suggests a tree-driven reduction of ^{13}C incorporation into the rhizosphere communities (−67%; Table 3). However, in the pine forest, ^{13}C uptake by trees remained almost unaffected by the moderate drought (−17%; Table 3). Nevertheless, the incorporation of ^{13}C-labeled assimilates by the rhizosphere communities (−72% for fungi) and rhizosphere respiration (−62%) were reduced, indicating that rhizosphere activity in the topsoil was suppressed by drought. A decrease in assimilate use by the rhizosphere implies that the microorganisms reduce the overall C use by trees and hence their sink activity, which may potentially feedback on C assimilation by trees (Gessler & Grossiord, 2019). However, additional studies are needed to quantify the importance of feedbacks between rhizosphere communities and trees.

High resilience of rhizosphere activity upon rewetting

Our finding of pronounced increases in the ^{13}C label in microbial communities in the rhizosphere after rewetting, to at least the same level as in continuously moist soils, in both forest ecosystem types (Fig. 3) signifies high resilience to drought of the functioning of rhizosphere communities in forests. In our study, we can exclude immediate impacts of rewetting on microbial communities, such as microbial cell lysis (Schimel *et al.*, 2007), as we conducted our ^{13}C pulse labeling experiments 2 wk after rewetting while such direct rewetting effects are confined to the first few days (Manzoni *et al.*, 2012). The primary reason for the high resilience seems to be the rapid recovery of belowground C allocation by trees following drought. This fast recovery apparently involved rapid phloem transport of assimilates from tree canopies to the rhizosphere a few days after rewetting, similar to observations in trees receiving a continuous water supply (Hagedorn *et al.*, 2016; Joseph *et al.*, 2020; Hikino *et al.*, 2022). Interestingly, in our study the ^{13}C incorporation into the microbial communities and rhizosphere respiration in rewetted (previously drought-exposed) soil often surpassed that observed for continuously moist soils in both forest ecosystem types (Fig. 3). Since ^{13}C uptake by trees under rewetting reached only the same level as that of continuously watered trees, the stronger ^{13}C label implies a preferred assimilate transfer and incorporation by rhizosphere communities, indicating their crucial role in whole-plant functioning. In support of this, the previous pulse labeling studies in the same mature pine forest revealed rapid recovery of belowground allocation (Joseph *et al.*, 2020; Gao *et al.*, 2021). In the young beech model ecosystems, there was an 'overshooting', that is greater belowground allocation upon rewetting in previously drought-exposed trees than in trees that had been irrigated continuously (Hagedorn *et al.*, 2016). The high priority of drought-exposed trees to investing in their rhizosphere during recovery from drought could be explained by the metabolic need of roots and mycorrhiza for water and nutrients acquisition (Hagedorn *et al.*, 2016; Hikino *et al.*, 2022). We assume that the overshooting after rewetting was only transient, as both forest systems experienced droughts in the years before our study and both showed suppressed ^{13}C transfer to their rhizospheres and their communities under drought (Fig. 2). Overall, the ^{13}C labeling experiments in the two forest systems give evidence that rhizosphere communities and trees are closely coupled and that this coupling can change within days in response to drying and rewetting, even in forests with trees more than 100 yr old.

Drought and rewetting effects on microbial substrate use

Our ^{13}C pulse labeling experiments indicate that drought and subsequent rewetting alter the functional use of rhizodeposits by microbial communities for growth, maintenance and osmoregulation. Under drought, there was a stronger decline of assimilates being incorporated into PLFAs than into microbial cytoplasm, especially for fungal 18:2ω6,9 and gram-negative bacteria (Fig. 5; Table S2). We interpret this pattern as a shift in allocation of fresh substrate by microorganisms under drought conditions, from processes related to growth to those linked to survival. Since PLFAs are components of cell membrane, their weaker ^{13}C signal in dry soils reflects reduced formation of new cells, which is indicative of suppressed cellular replication (Kaiser *et al.*, 2015). By comparison, microbial cytoplasm assessed as CFE-microbial biomass (Hogberg *et al.*, 2008; Lorenz *et al.*, 2021) consists primarily of compounds used for metabolic processes with high turnover rates (Malik *et al.*, 2016), for regulation of osmotic potentials (Karlowsky *et al.*, 2018a), and for storage (Mason-Jones *et al.*, 2023). Consequently, the lower ratio of the ^{13}C signal in PLFA to that in microbial cytoplasm under drought (Fig. 5) strongly suggests that rhizosphere microorganisms shifted their use of fresh rhizodeposits toward osmoregulation, storage and maintenance. These findings support those from a recent study by Mason-Jones *et al.* (2023), that microorganisms invest primarily in storage instead of replicative growth in times of reduced C supply (as under drought in our experiments). In our study, different microbial groups showed divergent drought responses regarding the ratios of the ^{13}C signal in PLFAs vs in microbial cytoplasm. The ^{13}C incorporation into fungi and gram-negative bacteria relative to the incorporation into microbial cytoplasm decreased under drought. By contrast, gram-positive bacteria showed a slightly stronger ^{13}C signal than microbial biomass, signifying that they may even profit from drought. Gram-positive bacteria have a thicker peptidoglycan cell membrane layer than gram-negative bacteria, providing them with greater resistance to drought (Schimel *et al.*, 2007; Manzoni *et al.*, 2012). Our results suggest that this gives gram-positive bacteria an advantage under drought, which supports DNA-based findings from the same mature pine forest showing a strong proportional enrichment of the gram-positive *Actinobacteria* under drought (Hartmann *et al.*, 2017).

Two weeks after rewetting, the ratios of ^{13}C in PLFAs vs microbial cytoplasm changed considerably in the young beech model ecosystems, with higher ratios in rewetted soils following drought than in continuously moist soils. This finding suggests a preferred allocation of fresh assimilates to new microbial cell membrane rather than to cytoplasm, which could be attributed either to microbial growth or to the repair of cell membrane upon rewetting (Amato *et al.*, 2010). By contrast, in the mature pine forest, the ratios remained lower in the dry control than in the irrigated plots after the rainfall event. One obvious explanation for this discrepancy is the lower intensity of the rewetting in the pine forest, which did not completely relieve drought conditions (Fig. 1). Alternatively, the long-term adaptation of the rhizosphere communities in the pine forest to repeated summer droughts, with an increasing contribution of oligotrophs (Hartmann *et al.*, 2017), might have caused smaller rewetting responses in terms of substrate use.

Differences between forest types and long-term effects

While the two ecosystem types showed similar patterns of ^{13}C incorporation into their rhizosphere communities during drought and after rewetting, the effects were larger in the young beech ecosystems than in the mature pine forest. We relate this finding primarily to the greater intensity of drought and rewetting in the beech model ecosystems compared with in the pine

forest, which experienced only natural rainfall events. Differences in tree size might have additionally contributed to the discrepancies in effect sizes. Specifically, the larger pine trees presumably stored greater amounts of C than the young beech trees, which could have caused a buffering of short-term changes in C assimilation, transport and use in the pine forest. In support of this latter explanation, the magnitude of the drought effect on ^{13}C uptake by trees, on photosynthesis, and tree nonstructural carbohydrate content was greater in the young beech ecosystems than in the mature pine forest (Hagedorn *et al.*, 2016; Schonbeck *et al.*, 2018; Joseph *et al.*, 2020).

In contrast to the incorporation of ^{13}C-labeled assimilates by the rhizosphere communities, the microbial community structure (as assessed by PLFA concentrations) remained unaffected by drought in the beech model ecosystems but showed significant responses in the pine forest. Specifically, the ratio of gram-positive to gram-negative bacteria was higher in dry soil than in irrigated soil in the pine forest (Table 2). We attribute the greater effect size in the pine forest to the longer experimental duration (15 vs 2 yr in the beech model ecosystems). The observed community shifts in the pine forest could have resulted either from long-term adaptation of the microbial communities to changes in soil moisture or from changed soil properties and substrate availability associated with the different water regimes. The latter explanation is supported by observation of an altered vertical distribution of soil organic matter (SOM) with long-term irrigation in the pine forest, with SOM losses in the organic layer but gains in mineral soil horizons (Guidi *et al.*, 2022). Our finding of increased ratios of gram-positive to gram-negative bacteria under drought in the pine forest suggests a shift toward communities using more recalcitrant SOM compared with labile plant-derived C (Fanin *et al.*, 2014). This is consistent with amplicon sequencing findings from the same pine forest, showing that the dry soils had greater abundances of bacteria with an oligotrophic life strategy than irrigated soils (Hartmann *et al.*, 2017).

Conclusions

Overall, the ^{13}C pulse labeling experiments in the two contrasting forest ecosystem types demonstrate that the incorporation of tree assimilates into rhizosphere communities is highly sensitive to drought and subsequent rewetting. (1) At the cellular level, the ^{13}C tracing show a shift in the microbial use of rhizodeposits under drought and rewetting. In dry soils, fewer rhizodeposits were incorporated into cell membrane, instead being preferentially transferred into the cytoplasm of the rhizosphere communities. This result suggests that new C from rhizodeposits was predominantly used for osmoregulation or storage, at the expense of new cell formation. Upon rewetting following severe drought in the beech model ecosystems, a greater proportion of new rhizodeposits was incorporated into microbial cell membrane, potentially to repair cells and regain microbial growth. (2) At the community level, fungi showed the strongest reduction in assimilate incorporation under drought among the microbial groups. (3) Despite the common response patterns of the two contrasting forest ecosystem types to drought and rewetting, the effect sizes of ^{13}C incorporation into the rhizosphere communities were greater in the beech model ecosystems experiencing stronger drought and rewetting. More studies in natural settings are required to fully understand the influence of the soil properties, tree species, and drought regime on the resistance and resilience of above- and belowground linkages under drought.

Acknowledgements

The experiment in the mature pine forest was conducted in the long-term irrigation experiment Pfynwald, which is part of the Swiss Long- term Forest Ecosystem Research Programme LWF (www.lwf.ch). This study was funded by the Sino Swiss Science and Technology Cooperation (EG09-122016; to DG and FH) and the Swiss National Science Foundation (SNF) under contract numbers 31003A_159866 and 310030_189109 (to AG). The authors are grateful to P. Bleuler for maintaining the infrastructure of both experiments, R. Siegwolf and C. Hug for their support in the pulse-labeling experiments, and U. Graf and A. Schlumpf for measuring stable isotopes. The authors are also thankful for the constructive comments from three anonymous reviewers and linguistic editing by M. Dawes. Open access funding provided by ETH-Bereich Forschungsanstalten.

Competing interests

None declared.

Author contributions

FH, AG and MA designed the study; FH, DG, JL, AG, AZ, MA, JJ, CP, EB, AR, MS and MH performed the pulse-labeling experiment and analyzed the samples; FH, DG, JL and RAW performed the data analysis; FH and DG wrote the manuscript with all authors contributing. DG and FH contributed equally to this work.

ORCID

Matthias Arend https://orcid.org/0000-0003-4514-0667
Edith Bai https://orcid.org/0000-0003-0495-6504
Decai Gao https://orcid.org/0000-0003-0545-4020
Arthur Gessler https://orcid.org/0000-0002-1910-9589
Frank Hagedorn https://orcid.org/0000-0001-5218-7776
Martin Hartmann https://orcid.org/0000-0001-8069-5284
Jobin Joseph https://orcid.org/0000-0002-6342-2505
Jörg Luster https://orcid.org/0000-0001-7048-212X
Christian Poll https://orcid.org/0000-0002-9674-4447
Andreas Rigling https://orcid.org/0000-0003-1944-4042
Marcus Schaub https://orcid.org/0000-0002-0158-8892
Roland A. Werner https://orcid.org/0000-0002-4117-1346

Data availability

The data that support the findings of this study are openly available in www.envidat.ch at https://www.doi.org/10.16904/envidat.477.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Experimental setup of the young beech model ecosystems used for the ^{13}C-pulse labeling during drought and 2 wk after rewetting.

Fig. S2 Distribution of scaffolds and the 10 ^{13}C pulse-labeled trees in dry control and irrigated plots in the mature pine forest.

Fig. S3 Principal component analysis and partial redundancy analysis of phospholipid fatty acids-based soil microbial communities in the young beech model ecosystems and in the mature pine forest.

Notes S1 Calculation of $\delta^{13}C$ values, $\Delta\delta^{13}CO_2$, and ^{13}C mass balance.

Table S1 Effects of drought and rewetting on the ^{13}C enrichment originating from ^{13}C pulse labeling.

Table S2 Statistical significance of the effects of experiment, drought and timing, as well as their interactions, on the ratio of $\Delta\delta^{13}C$ in PLFA to that in chloroform fumigation extraction-microbial biomass.

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