Intensive management disrupts belowground multi-trophic resources transfers in response to drought

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Article

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Intensive management disrupts belowground multi-trophic resources transfers in response to drought

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Abstract

Modification of soil food webs by historical land management may alter the response of ecosystem processes to climate extremes, but empirical support for this is limited and the mechanisms involved remain unclear. Here, we quantified how historical grassland management modifies transfers of recent photosynthate and soil nitrogen through plants and soil food web in response to drought, using *in situ* ^{13}C and ^{15}N pulse-labelling in paired intensively and extensively managed fields. We show that intensive management decreased plant carbon capture, its transfer through key components of food webs and soil respiration compared to extensive management. Drought only affected carbon transfer pathways in intensively managed grasslands, by increasing plant C assimilation but decreasing its transfer to plant roots, bacteria and Collembola. However, drought lowered the reduction of added nitrate to nitrous oxide in extensively managed grassland only. Our findings indicate that intensive management disrupts fluxes of recent photosynthates belowground, which impaired resistance of this process in response to drought. By contrast, extensive grassland management provides a greater potential to buffer impacts to drought by promoting the transfer of recent photosynthate belowground. Our work highlights that capture and rapid transfer of photosynthate through multitrophic networks is a key process for maintaining grassland resilience to drought.

Introduction

All organisms within ecosystems are interlinked by energy flows in complex multitrophic networks, and changes in the network structure modify these energy flows¹. Theoretical evidence suggests that shifts in food web structure play an important role in regulating the stability of soil functions following perturbations and impair their ability to buffer future extreme climatic events^{2–5}. Grasslands are under threat from ongoing degradation caused by multiple co-occurring drivers, including management intensification and climate extremes⁶. Drought events are a recurring phenomenon in many ecosystems and are predicted to increase in frequency and intensity in the coming decades^{7,8}. Consequently, there is a need to understand the interactions between these different drivers to inform sustainability policy aimed at protecting the multiple ecosystem services that grasslands provide^{6,9}.

Intensive grassland management, characterised by the regular use of inorganic fertilisers and high livestock stocking densities, is known to decrease plant diversity¹⁰, decrease the abundance and diversity of arbuscular mycorrhizal (AM) fungi¹¹ and soil biota^{12,13}, and induce shifts in the composition of soil microbial communities^{12–14}. Such changes have important consequences for biogeochemical cycles because soil food webs associated with agricultural intensification, including shifts in the relative abundance of bacterial and fungal energy channels, are often linked with faster nutrient mineralisation rates, which could potentially contribute to greater losses of carbon (C) and nitrogen (N) from soil^{15–18}. A critical gap in our knowledge concerns how shifts in food web structure and the relative abundance of fungi and bacteria modulate transfers of C from plants to below ground pools and fluxes, and the capture of growth-limiting nutrients by plants from soil energy channels.

Recent empirical studies indicate that intensive management can decrease the resistance to drought of plant productivity and soil respiration^{19,20} of soil food web biomass¹⁹, and of C allocation to soil microbial communities²¹. However, it is becoming apparent that the stability of ecosystem functions in response to perturbations can only be understood if multiple trophic

levels and interactions among them are considered ^{22,23}. As such, there is a need to study the role of food web structure from a multitrophic perspective, including plants and belowground organisms, in modulating the response of processes of C and N loss to perturbations, such as drought. Furthermore, while there is growing awareness of the importance of rhizodeposition as a driver of belowground energy flow and the structure and functions of soil food webs ²⁴⁻²⁹, it remains unclear how the resilience of this process to perturbations, such as drought, responds to changes in land use. These two major gaps in our knowledge are essential to facilitate reliable predictions of soil C and N cycling and sequestration in response to dual pressures of agricultural intensification and increased frequency and intensity of climate extremes.

We hypothesised that : (1) extensive grassland management promotes the transfer of photosynthetic C from plants to AM fungi and the decomposer food web, thereby lowering C and N losses as greenhouse gases (CO₂ and N₂O) and buffering C and N fluxes against drought; and (2) intensive management disrupts this coupling of C flow from plants to the soil food web, leading to greater soil C and N losses as CO₂ and N₂O and reduced resistance of C and N fluxes to drought.

We tested these hypotheses by simulating summer drought using rainfall shelters on paired extensively and intensively managed mesotrophic grasslands across three geographically distinct locations in northern England. In both control and drought plots, plant communities and soil food web (microorganisms and mesofauna) biomass and composition were determined. To investigate the short-term allocation of N and recently assimilated C belowground, we pulse-labelled plants with ¹³C-CO₂, and soil with ¹⁵N-NO₃ at the end of the simulated drought. We traced the incorporation of ¹³C and ¹⁵N into plant shoot and roots, microbial biomarkers, soil mesofauna and soil CO₂ and N₂O fluxes over a 20-day period.

Materials and methods

101 *Field sites and experimental design*

102 The field experiment was carried out in 2016 across a series of mesotrophic grasslands located
103 in the Yorkshire Dales, northern England (mean annual temperature 7.3°C, mean annual
104 precipitation 1382 mm). Three geographically distinct sites were selected, each with adjacent,
105 paired grasslands (field) with a long term (>20 years) history of intensive or extensive grassland
106 management on the same soils and of similar topography (see Table S1 for more details).
107 Extensively managed grasslands had not received any additions of inorganic fertiliser and had
108 been grazed at low stocking densities by sheep or cattle (typically grazed at <1 livestock unit
109 $\text{ha}^{-1} \text{y}^{-1}$)³⁰. Intensively managed grasslands received >100 kg N $\text{ha}^{-1} \text{y}^{-1}$ and were grazed at
110 high stocking densities or for longer (typically grazed at 2-3.5 livestock unit $\text{ha}^{-1} \text{y}^{-1}$)³⁰, and
111 were cut for hay once a year³⁰. In general, plant communities of the extensively managed
112 grasslands were species-rich *Anthoxanthum odoratum*-*Geranium sylvaticum* hay meadows
113 (MG3 or subcategories), whereas plant communities of intensively managed grasslands were
114 species-poor and classified as *Lolium perenne*-*Cynosurus cristatus* (MG6, MG7, and
115 subcategories), according to the UK National Vegetation Classification³¹. The soils were all
116 humose loamy brown earth (pH ~5.47; 11.4 % total C; 0.76 % total N).

117 Drought was simulated by placing three transparent roofs (1.5 m * 1.3 m) alongside
118 delimited control plots in each field for 60 days (17-19 of May – 17-19 of July 2016) designed
119 to simulate a 100-year drought event³². In each plot a 40 cm metallic collar (hereafter
120 “subplot”) was inserted into the first 7 cm of soil before the treatment was initiated. At the
121 centre of this collar a smaller plastic collar of 11 cm diameter was inserted and vegetation
122 inside it was clipped to allow measurement of gas fluxes from the soil only. After the removal
123 of the shelters, each plot (control and drought) received four litres of water to release the
124 drought and encourage photosynthetic activity and the isotopic labelling started as detailed
125 below.

126

127 Isotopic labelling

128

129 Figure 1. Conceptual diagram of the grassland soil food web of this study with representation
 130 of the expected flow of ^{13}C (blue) and ^{15}N (yellow) through plants and trophic groups. The
 131 arrows represent resource transfer between trophic groups. The isotopic enrichment is expected
 132 to decrease at higher trophic levels of the food web. Pictures of soil mesofauna from P. Lebeaux
 133 (www.animales.com) and M. Chomel.

134

135 We followed the movement of ^{13}C and ^{15}N into the plant shoots, through the soil
 136 community (microorganisms and mesofauna) and back to the atmosphere as CO_2 and N_2O
 137 emissions across a period of 20-days (Fig. 1). Five hours after the end of the drought, 130 ml
 138 of a solution of $\text{NH}_4\text{-}^{15}\text{NO}_3$ (Nitrate- ^{15}N , 98 atom%; CK Isotopes Ltd, Leicestershire, UK) was
 139 applied directly into the first 7 cm of soil with a side-hole needle by injecting into 20 locations
 140 within the subplots. The quantity injected was equivalent to the annual N deposition in the UK

(20 kg N ha⁻¹). Three hours later, a composite soil sample was taken consisting of 3 x 1 cm diameter cores from each subplot. The following morning, vegetation in the same subplots were labelled with 99 atom% ¹³C-CO₂ (Sigma aldrich). We used an air-tight chamber constructed with a plastic bell cloche (approx. 20 L) equipped with 2 small fans to disperse the gas, and a rubber septum at the top to inject the gas. Prior to the start of the ¹³C labelling ca. 10:00 GMT, the photosynthetic rate was measured using an infrared gas analyser (EGM-4, PP Systems, Hitchin, UK) to determine the timing of the CO₂ injections. During approximately 2-3 hours, 25 mL of ¹³C-CO₂ were regularly injected through the septum for a total of 250 ml per subplot. The twelve subplots within a site (i.e. both extensive and intensive management regimes) were labelled at the same time. The three sites were labelled over 3 consecutive days for logistical reasons.

Immediately after the ¹³C-labelling, a small subsample of plant shoots (ca. 0.5 g), 3 small soil cores (1 cm diameter), and gas samples were taken from each plot. At 1, 2, 5, 10 and 20 days after the ¹³C-CO₂ pulse labelling, gas samples were taken and a fifth of the soil from the subplots (metal ring) was harvested (Figure S1). We refer to day 1 as being approximately 24h after ¹³C labelling and 36h after ¹⁵N addition. The holes created were filled back with sand to minimise gas exchange from the exposed surface and disturbance. Four supplementary cores outside the plots were taken per field to determine the ¹³C and ¹⁵N natural abundance signatures of each C and N pool.

Plants were divided into shoot and root fractions, washed (for the roots fractions only), oven dried at 60°C and weighed prior to analysis. All samples were analysed for total C and N content and δ¹³C and δ¹⁵N signatures using an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd, Crewe, UK) coupled to a 20-20 isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK). A portion of soil was sieved and freeze dried prior to the PLFA extractions. From

the remaining soil, mesofauna were extracted using Tullgren funnels over 7 days and stored in 70% ethanol.

Soil microbial community analysis

Soil microbial communities were characterised by the extraction of the phospholipid fatty acids (PLFAs), according to Buyer and Sasser method ³³. Details of the method can be found in Chomel et al. (2019). The $\delta^{13}\text{C}$ signatures of individual PLFAs and their quantification were analyzed by GC–C–IRMS using a GC Trace Ultra with combustion column attached via a GC Combustion III to a Delta V Advantage IRMS (Thermo Finnigan, Bremen, Germany; Thornton et al. 2011). The internal standard 19:0 phosphatidylcholine (Avanti Polar Lipids) added at the beginning of the extraction procedure was used for calculating concentrations. In summary, 36 PLFAs were identified in these samples, of which 20 microbial specific PLFAs comprising approximately 80 % of the total concentration were used in subsequent data analysis. The fatty acids i15:0, a15:0, i16:0 and i17:0 were used as biomarkers for Gram positive bacteria; 16:1 ω 7, 18:1 ω 7, cy17:0 and cy19:0 were used as biomarkers of Gram-negative bacteria; and 15:0, 17:0 were used as general bacterial markers ³⁴. The fatty acids 10Me17:0 and 10Me18:0 were used as specific biomarkers of actinomycetes, and 17:1 ω 8c and 19:1 ω 8 were used as biomarkers of methane oxidizing bacteria ³⁴. Gram-positive, Gram-negative, and general bacterial markers were summed to give total bacterial PLFA. 18:2 ω 6,9 was used as a marker of fungi (Bååth, 2003; Bååth and Anderson, 2003) and 16:1 ω 5 was used as a marker of AM fungi ³⁵. Although 16:1 ω 5 is used widely for estimating AM fungal biomass, its use can lead to uncertainties because bacteria can contribute to this pool ³⁵. However, in our case, the ^{13}C -enrichment of this PLFA was very distinct from all bacterial PLFAs, which gave confidence in its use to estimate AM fungal biomass and its ^{13}C uptake. The $\delta^{13}\text{C}$ value of each PLFA molecule was corrected for the C added during derivatization using the formula

$$\delta^{13}C_{PLFA} = \frac{C_{FAME} \times \delta^{13}C_{FAME} - C_{MeOH} \times \delta^{13}C_{MeOH}}{C_{PLFA}}$$

where C_{FAME} , C_{MeOH} , and C_{PLFA} denote the number of carbon atoms in the FAME, methanol, and PLFA, respectively, and $\delta^{13}C_{FAME}$ and $\delta^{13}C_{MeOH}$ are the measured $^{12}C/^{13}C$ isotope ratios of the FAME and methanol, respectively (methanol $\delta^{13}C = -29.3\%$). While the fungi are represented by only one PLFA, the bacterial community is represented by several PLFAs. To calculate an overall ^{13}C -enrichment (atom% excess) of bacterial PLFA, the net ^{13}C of all individual bacterial PLFA were summed and divided by the sum of the net C of all individual bacterial PLFA using the following equation:

$$Atom\%^{13}C_{BacterialPLFA} = \frac{\sum (C_{PLFAi} \times atom\%^{13}C_{PLFAi})}{\sum C_{PLFAi}}$$

Where C_{PLFAi} is the amount of carbon from individual PLFA and $atom\%^{13}C_{PLFAi}$ is the ^{13}C -enrichment of the individual PLFA.

201

202 *Mesofauna*

203 Individuals were counted and identified under a dissecting microscope to order and trophic
 204 group for Collembola³⁶ and Acari³⁷. Other invertebrates were separated according to taxa and
 205 trophic group. The biomass of each order/taxa was estimated by measuring the average body
 206 length (mm) per field, using the formula by Caruso and Migliorini³⁸ for the mites and by
 207 Ganihar³⁹ for the other orders. The samples were further grouped into 7 main trophic groups
 208 in order to have sufficient material to analyse ^{13}C and ^{15}N : detritivorous Collembola,
 209 detritivorous mites (oribatid, astigmata and prostigmata mites), annelids, other detritivorous
 210 (detritivorous coleoptera, myriapoda and diptera larvae), herbivores (hemiptera and
 211 thysanoptera) predaceous mites (mesostigmata and predaceous prostigmata) and predaceous
 212 fauna (arachnida, chilopoda, predatory coleoptera and symphyla). Each of these groups was
 213 transferred into a tin capsule in 70 % ethanol, oven-dried and weighed prior to analysis. All the

samples were analysed for total C and N content and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a Flash EA 1112 Series Elemental Analyser connected via a ConFlo III to a DeltaPlus XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany).

Gas samples

Gas samples were taken by placing a 1.2 L dark chamber over the gas sampling core. Immediately after the closure of the chambers, and after 10, 20, and 30 minutes, 15 mL gas samples were taken from the headspace of the chamber using a gas-tight syringe fitted with an SGE syringe valve and transferred into pre-evacuated 12 mL gas-tight vial (Labco Ltd. UK). At the last time point, an additional 120 mL gas sample was taken and stored in an He-flushed, pre-evacuated 120 mL gas bottle fitted with Silicone/PTFE septa (Supelco) for ^{15}N - N_2O analysis. CO_2 and N_2O concentrations were analysed on all samples in a gas chromatograph 7890A GC (Agilent Technologies, USA) configured with a single channel using two detectors, an FID and a micro-ECD. The $\delta^{13}\text{C}$ values of the samples from the last sampling point were analysed on a Picarro G2131-*i* Isotope and Gas Concentration Analyser (cavity ringdown spectrometer). The $\delta^{15}\text{N}$ values of the samples from the last sampling point were analysed using a Sercon Ltd isotope ratio mass spectrometer following cryofocusing in an ANCA TGII gas preparation module (Sercon Ltd, Crewe, UK).

^{13}C and ^{15}N calculations

The isotopic concentration data was converted from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) to atom % excess ^{13}C and ^{15}N by subtracting the atom % ^{13}C and atom % ^{15}N of unlabelled controls from each enriched sample⁴⁰. ^{13}C - and ^{15}N -enrichment is independent of the pool size and is indicative of the replacement of C or N from the pool by newly incorporated plant-derived ^{13}C or fertiliser-derived ^{15}N .

Although the plants were pulse-labelled with the same amount of ^{13}C - CO_2 , differences in photosynthetic and respiration rates caused the initial amount of ^{13}C fixed to differ among subplots. To compare the relative transfer of ^{13}C from the plant shoot into belowground C pool (roots, microorganisms and soil fauna) in the different systems, the results were expressed as a percentage of the initial plant shoot ^{13}C enrichment.

The net incorporation of the ^{13}C or ^{15}N tracer into the different carbon/nitrogen pools for each subplot was calculated as:

$$\text{Net}^{13}\text{C pool} = \text{C pool} \times \text{atom \% } ^{13}\text{C pool}$$

where C pool is the amount of C in each pool (gC per subplot) and atom% ^{13}C pool is the atom % excess of ^{13}C of each pool. The same equation is used for the net incorporation of the ^{15}N , replacing ^{13}C and C by ^{15}N and N. In order to evaluate the recovery of the stable isotopes in the system, we calculated the fraction of the isotope in the different compartments as the ratio of the net incorporation amount in a compartment relative to the total initial uptake of ^{13}C by the plant shoot or the total ^{15}N label injected in the soil. These were calculated only for the relevant time point for which the enrichment was maximal: at day 1 for the plant and microorganisms and at day 5 for the soil fauna.

Data analysis

All analyses were performed using R software (version 4.1.0. R core Team, 2017). To assess the role of land management in driving the soil properties and plant biomass, we performed a principal component analysis (PCA). Principal coordinates analysis (PCoA) was applied to estimate the soil community structure using Bray–Curtis dissimilarities based on Hellinger transformed community data ⁴¹. A permutational multivariate analysis of variance (PERMANOVA) ⁴² was performed with 999 permutations to assess whether there were significant differences among grassland management. Distance comparisons in

PERMANOVA can be sensitive to between-group differences in dispersions, so we used PERMDISP⁴³ to determine whether the dispersions of each group around their group centroid were significantly different from one another.

To analyse the effect of land management on plant and soil organisms biomasses we used a mixed model with time and site as crossed random effects on data from control plots only⁴⁴.

To analyse the effect of land management on the ¹³C- and ¹⁵N-enrichment we used a mixed model with site as random effect and allowed different variance for each time point (with the function `weights=varIdent(1|time)`).

To quantify drought impacts, and its interaction with grassland management, we used an effect size calculated as the log Response Ratio (logRR), which quantifies the proportional difference between mean ¹³C- or ¹⁵N-enrichment in control and drought conditions⁴⁵. On a log scale, an effect size of 0 means there is no difference, a positive value means a positive effect of drought, while a negative logRR means a negative effect of the drought. To increase robustness, and because the trend of the response variable over time was relatively similar, we used the average value of each replicate over time in our calculation of logRR. We then performed a t-test against a mean of 0.

Results

1) Grassland management, soil food web composition and soil functioning

Land management modified plant communities and soil properties, with greater aboveground plant biomass, soil pH, nitrate concentration, and bulk density, and lower water holding capacity and belowground plant biomass in intensively managed grasslands compared to extensively managed grasslands (Fig S2). The influence of land management on soil CO₂ efflux was not consistent through the sampling period (interaction management*time, F=2.86, P =

0.0162, Fig S3), with higher CO₂ efflux in extensively managed compared to intensively managed grassland at time 0 and the opposite at day 20 (Fig S3). There was a trend of higher soil N₂O efflux in extensively managed grassland compared to intensively managed grassland on the first day after the pulse labelling, but this was not significant (interaction time*management, $P=0.08$, Fig S3)

Figure 2. Principal coordinates analysis (PCoA) of soil food web communities from grasslands with different land management (extensive in orange, intensive in blue). The PCoA was based on Bray-Curtis distance using soil mesofauna abundances (individuals m⁻²) and PLFA biomass (g m⁻²). Grey arrows show the correlation vectors of the variables with the ordination. Significance was tested by Monte Carlo permutation against 999 random datasets and variables with $P > 0.05$ were kept (see table S2).

Collembola was the most abundant group ($30,949 \pm 3,718$ ind.m⁻², 80% were within the entomobryomorpha group), followed by detritivorous mites ($25,114 \pm 2,772$ ind.m⁻², 88% were oribatids) and predatory mites ($20,094 \pm 2,279$ ind.m⁻²). Soil community composition differed significantly between extensive and intensive grassland (PERMANOVA, $F = 5.12$, $P < 0.001$). Despite this finding, there was substantial overlap in community composition (Fig. 2) and we detected significant differences in the distribution of data, with more dispersion in soil communities in extensive grassland (PERMISP, $F = 9.1$, $P = 0.033$). With the soil fauna pooled by trophic groups, the biomass of detritivorous mites (mainly Oribatids) was smaller ($F = 7.03$, $P = 0.0096$), while the biomass of other decomposers (mainly diptera larvae) was greater ($F=9.08$, $P = 0.0034$) in intensively managed compared to extensively managed grasslands (Figure S4). Intensive management did not have a significant impact on bacterial, fungal, AM fungal and actinobacteria biomass, nor the fungal/bacterial ratio across grassland sites (Fig S4).

2) *Effects of land management on ¹³C and ¹⁵N flow*

Figure 3 Temporal dynamics of ^{13}C in the plant shoot (upper panel), CO_2 (middle panel) and belowground pools (lower panel) following ^{13}C pulse labelling of plant shoots (time 0). The heat map shows the ^{13}C -enrichment in different pools and fluxes in grassland under extensive (left) or intensive (right) management. The plant shoot enrichment is expressed in atom% excess; to be able to compare the flux of C from the plant to belowground, all the other variables are expressed relative to the initial ^{13}C fixed by the plants (relative ^{13}C enrichment, %). Note difference in scales to visualise the relative enrichments. Results of the effect of the grassland management are reported with *** $P < 0.01$, ** $P < 0.01$, * $P < 0.05$, see Table S2 for details. Grey colour indicates no data available.

Figure 4 Temporal dynamics of ^{15}N atom % excess aboveground (upper panel) and belowground (lower panel) in plant and soil fauna following ^{15}N pulse labelling. The heat map shows the ^{15}N atom % excess in the different groups in grassland under extensive (left) or intensive (right) management. The two panels have different scales as the enrichment of aboveground nitrogen pools and fluxes is substantially greater than any of the below ground pools. There were no significant effects of grassland management: see Table S3 for statistical results. Grey colour indicates no data available.

Plant leaves in the intensively managed grassland were significantly less enriched in ^{13}C , consistently across the three sites, than in extensively managed grassland (Fig. 3, $P = 0.015$). The maximum ^{13}C -enrichment in plant shoots occurred at the end of the labelling period and was (on average) 0.55 and 0.39 atom% excess in extensively and intensively managed grassland, respectively. The enrichment decreased substantially 1 day after labelling to an

average of 0.13 and 0.11 atom% excess (Fig. 3). The uptake of ^{15}N in plant shoots was unaffected by grassland management, but increased gradually over two days after the labelling and reached a plateau of 4.5-5 atom % excess (Fig 4). Land management had no detectable influence on the ^{13}C - or ^{15}N -enrichment of plant roots (Fig. 4 and 5).

Among microorganisms, fungi had the highest enrichment of ^{13}C , whereas among soil fauna, Collembola had the highest enrichment of ^{13}C (Fig. 3). ^{13}C enrichment was greatest in AM fungi and detritivorous mites in extensively managed compared to intensively managed grassland (Fig 3, $P < 0.05$, see Table S3). No management effects on ^{15}N enrichment of soil fauna were detected (Fig. 4, $P > 0.05$, see Table S3). Although there was a greater ^{13}C -enrichment of the soil CO_2 efflux in extensively managed compared to intensively managed grassland ($P < 0.001$, Fig 4, Table S3), no difference in ^{15}N -enrichment of N_2O emissions was detected.

3) Effect of land management on the response of C and N flow to drought

During the rain exclusion, soil moisture was reduced on average by 56 ± 0.4 vol. % in intensively managed grassland and 74 ± 0.4 vol. % in extensively managed grassland over the last 27 days recorded (Fig S1).

In intensively managed grassland, the drought increased the biomass of microbial communities and of detritivorous mites, but reduced the biomass of plant shoots and of other detritivores (Fig. S5). In extensively managed grassland, the drought increased the biomass of detritivorous mites and predatory mites, but decreased the abundance of actinobacteria PLFA and biomass of Collembola (Fig. S5).

Figure 5. Response ratio of the drought effect on the ^{13}C (A) or ^{15}N (B) enrichment of the different pools in function of the grassland management (log (drought/control)). The sign (positive or negative) of the logRR corresponds to the direction of the drought effect on the ^{13}C or ^{15}N enrichment, while a response ratio of zero indicates no drought effect. LogRR significantly different from 0 are reported with *** $P < 0.01$, ** $P < 0.01$, * $P < 0.05$, see table S4 for details.

In extensively managed grassland, drought had no detectable effect on the uptake of ^{13}C by plants, its transfer to roots and the soil food web, or soil ^{13}C - CO_2 efflux (Fig. 5, Table S4). However, in intensively managed grassland, drought increased plant shoot ^{13}C enrichment (Fig. 5, Table S4. $P = 0.004$) and ^{13}C relative enrichment of soil CO_2 efflux (Fig. 5, table S4, $P = 0.032$), but decreased its transfer to roots, bacteria and Collembola (Fig. 5, table S4, $P = 0.026$, 0.023 , 0.0048 , respectively). Drought had no impact on N fluxes to the plants and soil communities, and only reduced ^{15}N transfer as N_2O efflux in extensively managed grassland compared to the control (Fig. 5, table S4, $P = 0.023$).

4) ^{13}C and ^{15}N allocation

One day after labelling, 26 % of ^{13}C fixed by plants remained in the shoots and 2.6 % was recovered in the roots (Fig S6). A similar pattern was observed with the ^{15}N tracer, for which 39 % was on average recovered in the plant shoots and 12% in the plant roots (Fig S6) one day after the labelling. Intensive management decreased the ^{13}C recovery in plant roots but increased the ^{15}N recovery in plant shoots (Fig S6, Table S5).

Analysis of ^{13}C and ^{15}N pools revealed that in extensively managed grassland, soil fauna tended to store more photosynthesized carbon and fertiliser-derived N compared to intensively managed grassland (Fig S7). This pattern becomes even clearer when considering the relative allocation (Fig S6, panel C). Indeed, extensive management increased the ^{13}C recovery in AM fungi, detritivorous mites and predatory mites, and the ^{15}N recovery in detritivorous mites (Fig S6, Table S5, $P < 0.05$). Drought only decreased the recovery of the ^{15}N in the plant roots (Fig S6, Table S5, $P < 0.001$) and had no significant effect on the recovery of the tracers in other C and N pools (Fig S6, Table S5, $P > 0.05$).

Figure 7. Schematic of the influence of grassland management (left panel extensive, right panel intensive) and drought (negative impact in red, positive impact in violet, and no significant impact in black) on ^{13}C uptake by plant shoot and transfer to roots, soil organisms and CO_2 efflux, and ^{15}N transfer to N_2O efflux. The size of the arrows corresponds to the relative proportion of ^{13}C or ^{15}N incorporated by the plant, soil organisms and released as soil efflux. Pictures by M. Chomel, illustrations by J. Bardgett.

Discussion

Our findings on the reciprocal flow of C and N through plants and soil food webs shed new light on our understanding of how land management substantially modifies the response of multitrophic networks to drought, with consequences for the key ecosystem processes they regulate. We found that drought strongly affected fluxes of recent photosynthate belowground

in the intensively managed grassland, indicating impaired resistance of this process, likely through a decoupling of above-below ground interactions (Fig. 6). In contrast, belowground fluxes of recent photosynthate in extensive grasslands were unaffected by drought, indicating greater potential to buffer impacts of climate extremes on above-below ground interactions.

The effect of drought on plant C assimilation and its allocation belowground differed between the two grassland management types. Despite decreases in aboveground biomass, drought increased plant shoot C uptake and decreased C transfer to roots in intensively managed grassland, but had no impact on these C transfers in extensively managed grassland. This finding is consistent with recent reports of greater resistance and faster recovery of plants in abandoned relative to managed grasslands due to larger belowground root and fungal networks in the former, which improves water access compared to intensively managed grasslands^{19,21,46}. In our study, the greater uptake of C by plants in intensively managed relative to extensively managed grasslands could be a compensatory effect following the release of the drought. Indeed, fast-growing plants, which dominate the plant community of intensively managed grasslands, typically have an ability to open their stomata more quickly when drought is released compared to slow-growing plant species⁴⁷, which dominate the plant community in extensively managed grasslands. Our results confirmed that intensive management reduces plant diversity and promotes species with lower root-to-shoot ratio. These differences are explained by the fact that intensive management promotes fast-growing plant species, which store resources in roots to facilitate regrowth after cutting, while extensive management promotes plants that invest in root growth, rather than storage, to access soil resources²¹. However, in our study, historic management intensity had no detectable effect on root ¹³C enrichment, indicating a similar rate of root C allocation of newly incorporated photosynthates in both management regimes, despite large differences in their biomass allocation.

Fungi and Collembola were a major conduit of recent photosynthate-derived C; on average 5.8 % of fungal C and 7.6 % of Collembola C came from plant photosynthate at their enrichment peak. The high ^{13}C -enrichment of non-mycorrhizal fungal PLFA compared to the AM fungal PLFA is surprising, and implies an important role of non-mycorrhizal fungi in channelling plant-derived C into the soil food web, supporting other recent findings^{48–51}, and reflecting that saprotrophic fungi form a significant portion (20–66 %) of microbial biomass in a grassland rhizosphere⁵². Although the recovery of the ^{13}C tracer was greater in bacterial PLFA compared to AM fungal or general fungal PLFA (see Fig. S5), the conversion of PLFA to biomass is higher for fungi, and this means the absolute amount of ^{13}C in bacterial biomass was less than for fungal biomass (11.8 nmol of the PLFA 18:2 ω 6,9 = 1 mg of fungal C while 363.6 nmol of total bacterial PLFA = 1 mg bacterial C). A greater proportion of plant-derived C was recovered in AM fungi in extensively managed compared to intensively managed grassland, despite similar biomass in the two systems. This pattern may be due to enhanced demand or efficiency of C uptake by AM fungi in extensive relative to intensive managed grasslands. Similar results have been found for non-mycorrhizal fungi during the course of nature restoration on abandoned arable land, where fungal biomass was not impacted, but enhanced efficiency of C uptake by the fungi was observed during nature restoration⁵⁰. Our results show that the fungal energy channel has a more important role in extensively managed grassland and promotes the retention of recently assimilated C in soil^{19,48}.

Drought decreased the flow of ^{13}C through several components of the food web, notably bacteria and Collembola. Collembola are more sensitive to drought than other soil fauna and often reduce their grazing activity in response to drought²⁹. Bacteria grow quickly and are more sensitive to drought and other stresses than fungi^{53–58}. Furthermore, fungi are able to create

large hyphal networks that facilitate nutrient and water transfer over long distances, and indirectly benefit plants by exploring water-filled soil pores not accessible to plant roots^{57,59,60}.

Our findings also indicate that soil activity (including roots and soil organisms) depends less on C derived from recent photosynthate in intensively managed compared to extensively managed grasslands. This finding can be explained partially by the fact that there is less root biomass, and so less C input belowground, in intensive systems. Furthermore, our results show that there is less plant-derived C transfer to AM fungi and oribatid mites in intensively managed grassland, supporting the idea that intensive management modifies food web structure and decreases the flow of energy through AM fungi^{2-5,61}. Rewetting at the end of the drought period led to an increase of respiratory losses of recent C from the ecosystem in intensively managed grassland. During drought, C pools may accumulate and become metabolically available for roots and microbes upon rewetting⁴⁶. In addition, microbes can become potentially active after drought within hours upon rewetting⁶²; they can resuscitate from dormancy^{53,63}, and depending on the duration of drought, they can start to regrow within one to several days⁶⁴⁻⁶⁶.

In extensive grassland there was marginal greater emission of ¹⁵N-N₂O (which we attribute primarily to the nitrate reducing processes of denitrification and nitrate ammonification) immediately after the ammonium nitrate was injected into the soil and during the following day (Figure S3). This contradicts the general idea that intensive management of soil enhances the production of N₂O^{67,68}. This is likely to reflect the higher C availability in extensively managed grassland providing the reductant for more sustained nitrate reduction⁶⁹. Moreover, in extensive grassland, drought did not modify N₂O efflux, but it decreased its ¹⁵N-enrichment compared to the control (in intensive grassland, there was a significant effect at day 1 only). Soil moisture has consistently been shown to be one of the most important parameters affecting soil oxygenation and thus determining N₂O production rates^{70,71}, and may

change the microbial source of measured N_2O ⁷². Under drought, higher concentrations of O_2 in soil pores may favour ammonia oxidation of unlabelled NH_4 resulting in ^{14}N -dilution of the $^{15}\text{N}\text{-NO}_3$ pool, and of the subsequent N_2O emission. Surprisingly, ^{15}N transfer to plants and soil organisms was unaffected by land management and drought, suggesting that soil driven processes of resource transfer are more buffered against perturbations compared to plant-driven processes.

Conclusion

Our findings show that intensive land management impaired the resistance of plant-derived C flow to drought, likely through a decoupling of above-below ground interactions. Moreover, our study demonstrates how the interplay between land management and climate extremes regulates bidirectional flows of nutrients through multitrophic networks. Surprisingly, grassland management and drought had little influence on the soil N flow, and N_2O emissions were only higher after the drought in extensively managed grassland. By contrast, grassland management alters the ability of plants and soil organisms fuelled by recent photosynthate to buffer the response of C process to an extreme event. Our findings demonstrate that capture and rapid transfer of photosynthate through multitrophic networks is a key process for maintaining grassland resilience to drought. This highlights the need for future studies to examine trade-offs of grassland management intensities for various climate change scenarios (e.g. drought), which will identify resilient grassland management practices that can deliver sustainable food security in the face of climate change.

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Author contribution

MC, JL, FdC, RDB, DJ, ME, TC, FdV and EB conceived and designed the experiment. MC, JL, FdC, NA, JR and MM set-up the main experiment, MC and NA performed the pulse labelling and field sampling. MM identified soil fauna, MC, NA, and HS performed laboratory analyses. MC statistically analysed the data and led writing the manuscript in close consultation with DJ, TC and RDB and with discussions and contributions from all authors.

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