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Drying and rewetting effects on soil microbial community composition and nutrient leaching

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Abstract

The effects of a dry-rewetting event (D/RW) on soil microbial properties and nutrient release by leaching from two soils taken from adjacent grasslands with different histories of management intensity were studied. These were a low-productivity grassland, with no history of fertilizer application and a high-productivity grassland with a history of high fertilizer application, referred to as unimproved and improved grassland, respectively. The use of phospholipid fatty acid analysis (PLFA) revealed that the soil of the unimproved grassland had a significantly greater microbial biomass, and a greater abundance of fungi relative to bacteria than did the improved grassland. Soils from both grasslands were maintained at 55% water holding capacity (WHC) or dried to 10% WHC and rewetted to 55% WHC, and then sampled on days 1, 3, 9, 16, 30 and 50 after rewetting. The D/RW stress significantly reduced microbial biomass carbon (C), fungal PLFA and the ratio of fungal-to-bacterial PLFA in both soils. In contrast, D/RW increased microbial activity, but had no effect on total PLFA and bacterial PLFA in either soil. Microbial biomass nitrogen (N) was reduced significantly by D/RW in both soils, but especially in those of the improved grassland. In terms of nutrient leaching, the D/RW stress significantly increased concentrations of dissolved organic C and dissolved organic N in leachates taken from the improved soil only. This treatment increased the concentration of dissolved inorganic N in leachate of both soils, but this effect was most pronounced in the improved soil. Overall, our data show that D/RW stress leads to greater nutrient leaching from improved than from unimproved grassland soils, which have a greater microbial biomass and abundance of fungi relative to bacteria. This finding supports the notion that soils with more fungal-rich communities are better able to retain nutrients under D/RW than are their intensively managed counterparts with lower fungal to bacterial ratios, and that D/RW can enhance nutrient leaching with potential implications for water quality.

Keywords: Grassland; Carbon; Dissolved organic carbon; Dissolved organic nitrogen; Dissolved inorganic nitrogen; Drying and rewetting; Leaching; Leachates; Microbial biomass; Nitrogen; PLFA; Nutrient retention and release

1. Introduction

There is growing recognition that environmental stressors and perturbations have marked effects on microbial physiology and community composition, with implications for energy and nutrient flows in terrestrial ecosystems (Schimel et al., 2007). One key example of this is soil drying and rewetting (D/RW), which, subjects soil microbes to physiological stresses by decreasing substrate diffusion leading to changes in metabolism (Stark and Firestone, 1995; Schimel et al., 2007). Drying and rewetting can also alter soil water potential creating osmotic stress (Halverson et al., 2000), leading to microbial death and cell lysis (Bottner, 1985; Turner et al., 2003) unless they are able to resist the stress (Griffiths et al., 2003) or become dormant until conditions become more favourable (Schimel et al., 2007). Ultimately, all these processes occur simultaneously and, given that soil microbes act as a sizable and temporally dynamic sink for nutrients in terrestrial ecosystems (Zogg et al., 2000; Bardgett et al., 2003, 2005;

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Olander and Vitousek, 2004), can have implications for nutrient cycling and loss from soil via leaching to ground and surface waters (Turner and Haygarth, 2001).

Many studies have shown that soil D/RW events can cause an initial, short-lived, flush of C, N and phosphorus (P) into soil solution, and an increase in soil respiration rate, indicating enhanced microbial activity. There are several explanations given for the effects of soil D/RW events in the literature. First, soil aggregate disruption and cracking of organic colloids releases soil organic matter (SOM) protected within micro- and macro-aggregates, increases the extractability of the non-biomass SOM and exposes new soil surfaces for microbial attack (Powlson and Jenkinson, 1976; Adu and Oades, 1978; van Gestel et al., 1991; Appel, 1998; Lundquist et al., 1999; Magid et al., 1999; Fierer and Schimel, 2003; Miller et al., 2005). It also allows these recalcitrant nutrients to be transformed into a more labile nutrient pool (Lundquist et al., 1999; Wu and Brookes, 2005) contributing to the flush of nutrient mineralization and solubilization. Second, nutrient flushes following D/RW events have been attributed to microbial cell lysis, which releases nutrients into soil solution which were previously contained within microbial cells, many of which being organic (Bottner, 1985; van Gestel et al., 1993a, b; Grierson et al., 1998; Magid et al., 1999; Turner and Haygarth, 2001; Turner et al., 2003; Wu and Brookes, 2005). Finally, soil drying subjects microorganisms to very low water potentials, and under these conditions microbes need to lower their water potential to be in equilibrium with the environment around them. To achieve this they accumulate intracellular organic and inorganic solutes (Halverson et al., 2000). Rewetting of soil after a drying event creates an extreme stress, increasing soil water potentials requiring microbial cells to increase their water potential by rapidly releasing these solutes to again achieve a state of equilibrium (Halverson et al., 2000; Fierer and Schimel, 2003).

While previous studies have examined how the soil microbial biomass responds to D/RW, it is not known how differences in soil properties, including variations in microbial community structure, influence the ability of soils to retain nutrients after perturbations brought about by changes in soil moisture conditions, i.e., hydrological pulsing or D/RW. In this study, we examined how differences in long-term grassland management, and associated changes in soil microbial community composition, influence the ability of soils and the soil microbial biomass to retain or leach nutrients after D/RW perturbations. We focus on grasslands because previous studies have shown that soil microbes act as a significant and highly dynamic sink for nutrients in these systems (Bardgett et al., 2003; Harrison et al., 2007) and long-term differences in management intensity of grassland lead to marked differences in soil microbial community composition: unfertilized, low-productivity grasslands (referred to as unimproved) have a greater fungal relative to bacterial biomass than their intensively managed, fertilized (referred to as improved) counterparts (Bardgett and McAlister, 1999; Bardgett et al., 2001; Donnison et al., 2000; Grayston et al., 2001; Smith et al., 2003; de Vries et al., 2006). How soils with different microbial communities, nutrient status and soil structure, resulting from differing management histories, respond to D/RW remains unknown. To redress this, this paper tested the hypothesis that low productivity, unimproved grassland soils with a greater fungal relative to bacterial biomass are better able to retain nutrients under D/RW stress than are the lower fungal content communities of improved grassland. Overall, we anticipate that our data, although obtained from laboratory incubations, will provide insights into the way that changes in microbial community composition, and other soil properties such as soil moisture and nutrient status, might influence nutrient retention in soil and also has wider implications for understanding biotic processes that might control nutrient leaching to water.

2. Methods and materials

2.1. Site description and soil preparation

Soil was taken from two adjacent, sheep-grazed grasslands on similar parent material, but of different long-term management, located at Littledale, in north Lancashire, UK (54°3′N, 2°42′W). One had not received any fertilizer applications and was grazed at low stocking densities (1–2 ewes ha^{-1}), whereas the other had received regular applications of fertilizer (NPK) and farmyard manure, and was heavily grazed (10–15 ewes ha^{-1}). These grasslands are hereafter referred to as unimproved and improved, respectively. These long-term differences in management have led to different plant communities: vegetation of the unimproved grassland was classified as *Festuca-Agrostis-Galium* (UK National Vegetation Classification [NVC] U4a; Rodwell, 1992), while the improved grassland was a *Lolium-Cynosurus* grassland (NVC MG6; Rodwell, 1992). Both soils were brown earths (FAO classification: Cambisol); the soil of the improved grassland had a significantly higher pH (6.06 ± 0.04 [mean $\pm$ 1SE]) than the unimproved grassland (pH: 4.30 ± 0.04). Total soil C was greater in the unimproved ($8.0 \pm 0.3\%$) than improved ($5.3 \pm 0.2\%$) soil, as was total soil N (improved: $0.6 \pm 0.02\%$; unimproved: $0.7 \pm 0.02\%$). Previous studies of the site also showed that the unimproved soil had a greater biomass of fungi relative to bacteria than did the improved soil, which had a more bacterial-dominated microbial community (Bardgett et al., 2003; Grayston et al., 2004). The site is located at approximately 300 m altitude and the mean growing season temperature in this region is 15 °C, with a mean annual precipitation of 1200 mm. As noted above, differences in the soil and vegetation composition of the two sites are related entirely to long-term agricultural management (Bardgett et al., 2003; Grayston et al., 2004). Vegetation and soil properties of these sites at Littledale are representative of comparable sites that are widespread

throughout the upland regions of the UK, containing plant species that are common to most temperate, agricultural grasslands (Bardgett et al., 2003).

Four randomly selected bulk soil samples (~3 kg) were taken to 15 cm depth from each site in early March 2005. While still field-moist, the soil samples were coarsely sieved (5 mm), yielding four independent samples from each of the unimproved and improved sites. Prior to setting up the microcosm experiment, the soils were stored at 4 °C. Abiotic properties of the soil, such as pH, total C and N, and moisture content, were determined using standard protocol (Allen, 1989). Soil water holding capacity (WHC) was determined using the method described by Fierer and Schimel (2002).

2.2. Experimental design

A microcosm pot experiment was set up using subsamples (50 g dry weight equivalent per pot) of each of the four soil samples collected from the two grasslands. Four replicate pots were used for each treatment combination, which included: grassland type (unimproved vs. improved), moisture (D/RW vs. control) and sampling date (days 1, 3, 9, 16, 30 and 50 after rewetting (24, 72, 216, 384, 720 and 1200 h after rewetting)), totalling 96 microcosms. The first set was used for the determination of leachate nutrient release (dissolved organic carbon (DOC); dissolved organic nitrogen (DON); and dissolved inorganic nitrogen (DIN)), and analysis of microbial activity, measured as soil basal respiration, from intact microcosms; these were set up in gauze-based pots to facilitate the collection of leachates. An additional set of microcosms were also set up to allow destructive harvests at each sampling date for measurement of microbial biomass C and N; total C and N; phospholipid fatty acid (PLFA) extraction; and pH. Both sets of pots were treated identically prior to sampling and were sampled at a series of time points (days 1, 3, 9, 16, 30 and 50 after rewetting).

The moisture treatment was performed by air drying the D/RW soils to 10% WHC (soil water potentials of −4.77 MPa in the unimproved soil and −5.47 MPa in the improved soil) over a period of 10 days, while the control soils were maintained at 55%WHC (−0.18 MPa in the unimproved soil and −0.16 MPa in the improved soil), this stage was performed at room temperature ~20 °C. Once the dried soils reached 10% WHC, all of the microcosm pots were placed in a randomized block arrangement in an incubator at 15 °C (the average growing season temperature) for 46 h, with the control soils being maintained at 55% WHC during this period. The dried soils were then rewetted to 55% WHC and then all of the pots were returned to the incubator for 24 h prior to day 1 analysis. The pots were kept in the incubator until their respective sampling dates, other than when they were being watered to maintain the soils at 55% WHC.

Soil water potential was measured using thermocouple psychrometry, and plotted against gravimetric soil water content. Soil samples were placed in a pre-weighed psychrometer cup, covered with pre-weighed aluminium foil then the entire sample weighed. Sample cups were loaded into a C52 chamber (Wescor Inc, USA) and incubated for 1 h and voltage read with a HR-33T microvoltmeter (Wescor Inc, USA). Voltages were converted into water potentials based on calibration with salt solutions of known osmotic potential. After removal of the psychrometer cup, it was placed in a drying oven at 60 °C for 3 days and then weighed. Gravimetric water content of the sample was calculated as weight of water divided by weight of dry soil.

2.3. Soil microbial properties

Microbial biomass C and N were measured at each sampling date using the fumigation–extraction techniques of Brookes et al. (1985) and Vance et al. (1987). Briefly, soil samples (5 g at 55% WHC) were fumigated with $CHCl_3$ for 24 h at 25 °C. After the removal of the $CHCl_3$, soluble C was extracted from the fumigated and from un-fumigated samples with 0.5 M K_2SO_4 for 30 min on an orbital shaker (soil: solution 1:4 w/v). Total organic C (TOC) in filtered extracts (Whatman No. 1) was determined using a Shimadzu 5000A TOC analyser. Microbial C flush (difference between extractable C from fumigated and un-fumigated samples) was converted to microbial biomass C using a k_{EC} factor of 0.35 (Sparling et al., 1990). Extractable N in the above extracts was determined by oxidation with $K_2S_2O_8$ using the methodology of Ross (1992), and measurement of the resultant NO_3^--N and NH_4^+-N by auto-analyser procedures using a Bran+Luebbe Autoanalyser 3. The microbial N flush was converted to microbial biomass N using a k_{EN} factor of 0.54 (Brookes et al., 1985). Microbial activity was measured as basal respiration, using the method described by Bardgett et al. (1997). Prior to leaching, the pots (each containing 50 g dry weight equivalent of soil at 55% WHC) were placed in Kilner jars and incubated for 24 h at 15 °C. One millilitre headspace gas was then removed and CO_2 concentration was measured against a 1% standard gas on an infra-red gas analyser and expressed as $\mu l\, CO_2\, g^{-1}$ dry soil hr^{-1}.

Microbial community structure was assessed using PLFA analysis. This method was carried out on soils from day 30 only. This three-step procedure involved the extraction, fractionation and quantification of PLFAs, based on the method of Bligh and Dyer (1959), modified by White et al. (1979) and described by Bardgett et al. (1996). Separated fatty acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using standard qualitative bacterial and methyl-ester mix (Supelco) that ranged from C11 to C20. Fatty acid nomenclature was used as described by Frostegård et al. (1993). The fatty acids i15:0, a15:0, 15:0, 16:1ω7, i17:0, 17:0, cy17:0, C18:1ω9 and cy19:0 were chosen to represent bacterial PLFAs (Tunlid et al., 1989; Frostegård

et al., 1993; Frostegård and Bååth, 1996) and 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986; Frostegård and Bååth, 1996). In all samples, a total of between 27 and 30 individual fatty acid methyl esters were identified and represent total PLFA. For each sample the relative abundance of bacterial and fungal fatty acid methyl esters was expressed as the proportion (%) of the sum of all fatty acid methyl esters. The ratio of fungal-to-bacterial biomass was represented as the ratio of 18:2ω6: bacterial PLFA (Bardgett et al., 1996; Frostegård and Bååth, 1996).

2.4. Soil leachates

Leachates from each date were collected for the analyses of DOC, DON and DIN by flushing the gauze-based microcosm pots with 100 ml of double-deionized water. Total and inorganic C were determined using a Shimadzu 5000A TOC analyser, and DOC was then calculated by subtracting the amount of inorganic C from the total C in the samples. Total N was determined by oxidation with $K_2S_2O_8$ using the methodology of Ross (1992), and measurement of the resultant NO_3^--N and NH_4^+-N by auto-analyzer procedures using a Bran + Luebbe Autoanalyser 3. DON was then calculated by subtracting the amount of inorganic N from the total N in the samples.

2.5. Statistical analysis

The effects of the independent variables, namely sampling date, grassland type and D/RW, on soil microbial and nutrient properties were analysed using a three-way ANOVA with the SAS statistical package (SAS Enterprise Guide, 1999), with data being presented up to the two-way interaction level. Dependent variables were normalized, if required, prior to analysis using $\log_{10}$ transformations, this was done for data on leachate DOC. A square root transformation was used for microbial biomass N, microbial activity, and leachate DIN, and reciprocal transformations were carried out on data for leachate DON. Data on microbial biomass C and pH did not require transformation. PLFA data were analysed using a two-way ANOVA and square root transformations were required for total fungal PLFA; the ratio of fungal-to-bacterial PLFA data required $\log_{10}$ transformations; total PLFA data required reciprocal transformations; total bacterial PLFA data did not require transformation.

3. Results

3.1. Soil microbial properties

Soil D/RW reduced microbial biomass C (12%; $F_{1,48} = 9.25$, $P < 0.01$) and microbial biomass N (39%; $F_{1,69} = 58.34$, $P < 0.001$) in both grasslands (Table 1) over the experimental period. The reduction in microbial biomass N as a result of D/RW was greater in the improved than the unimproved grassland soil ($F_{1,69} = 4.86$, $P < 0.05$ for grassland × treatment interaction) (Table 1, Fig. 1). The effect of D/RW on microbial biomass N was especially pronounced early on in the experiment, as evidenced by a significant D/RW × sampling date interaction ($F_{5,69} = 9.64$, $P < 0.001$) (Table 1); on days 1 and 3, D/RW reduced microbial biomass N by 51% and 52%, respectively, relative to the control (Fig. 2). The main factor explaining variance in microbial biomass C was grassland type ($F_{1,48} = 87.79$, < 0.001) (Table 1), in that this measure was greater in the unimproved than in the improved soil (Table 2). Microbial biomass C also varied significantly ($F_{3,48} = 64.95$, < 0.001) over the experimental period (Table 1), being greatest on day 1 and lowest on day 16 (Table 2). Microbial biomass N was greater in the improved than the unimproved soil on day 3, but the

Table 1
Summary statistics of a two-way ANOVA looking at the effects of D/RW, grassland type and sampling date on microbial biomass C and N, microbial activity, total PLFA, total bacterial and fungal PLFAs measured as a % of total PLFAs and the ratio of % total fungal to % total bacterial PLFAs

	Biomass C			Biomass N			Microbial activity		Total PLFA		Total bacterial PLFA		Total fungal PLFA		% total fungal : % total bacterial PLFA	
	df	F	P	df	F	P	F	P	F	P	F	P	F	P	F	P
Main effect																
D/RW	1	9.25	<0.01	1	58.34	<0.001	22.22	<0.001	0.12	ns	1.79	ns	5.72	<0.05	5.78	<0.05
Grassland type	1	87.79	<0.001	1	0.00	ns	1.41	ns	46.85	<0.001	136.73	<0.001	20.34	<0.01	79.12	<0.001
Date	3	64.95	<0.001	5	67.56	<0.001	5.29	<0.001								
Two-way																
Grassland type × D/RW	1	0.09	ns	1	4.86	<0.05	1.09	ns	0.00	ns	0.81	ns	0.47	ns	1.57	ns
Date × D/RW	3	0.61	ns	5	9.64	<0.001	2.06	ns								
Date × grassland type	3	3.82	<0.05	5	3.92	<0.01	0.51	ns								
Residuals	48			69			72		12		12		10		8	

Values for microbial biomass N, microbial activity and % total fungal PLFA were normalised using square-root transformations, values for the ratio of % total fungal to % total bacterial PLFAs were normalised using a $\log_{10}$ transformation and values for total PLFA were normalised using a reciprocal transformation prior to analysis (ns indicates not significance at $P < 0.05$).

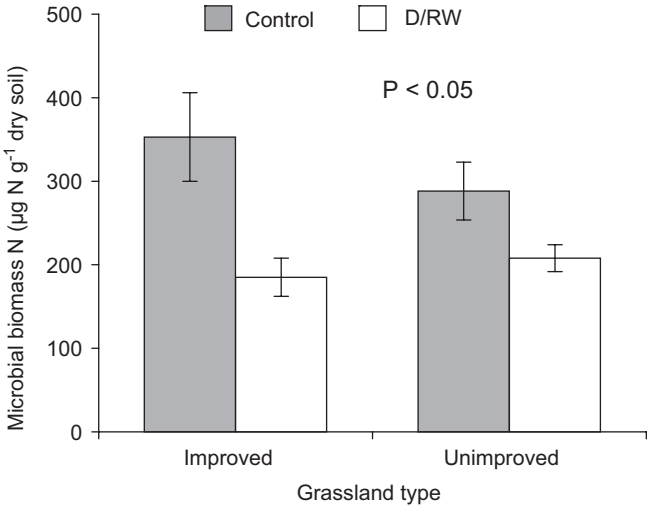

Fig. 1. Effects of the 'drying and rewetting × grassland type' treatment interaction on microbial biomass N. Bars represent means (±SE).

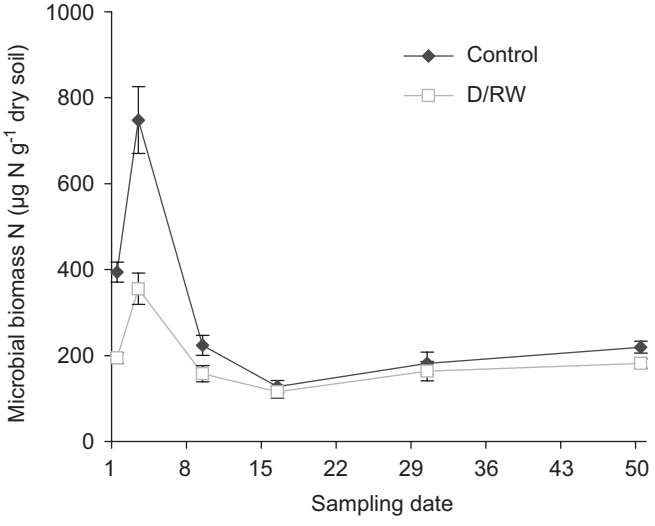

Fig. 2. Effects of drying and rewetting on microbial biomass N, over the experimental period. Bars represent means (±SE).

opposite was true on days 9 and 16 ($F_{5,69} = 3.92$, $P < 0.01$ for the grassland type × sampling date interaction) (Table 1; Fig. 3); there was no main effect of grassland type on this measure (Table 1). Microbial biomass N varied across sampling dates ($F_{5,69} = 67.56$, $P < 0.001$) (Table 1) being greatest on day 3 and lowest on day 16 in both soils (Table 2).

Microbial activity, measured as basal respiration, was influenced significantly by D/RW ($F_{1,72} = 22.22$, $P < 0.001$) (Table 1), being 53% greater in both soils relative to controls (Table 2). Microbial activity also varied significantly ($F_{5,72} = 5.29$, $P < 0.001$) (Table 1) across the sampling days, being greatest early on in the experiment on days 1 and 3 (Table 2). Microbial activity did not differ significantly between grassland types and was unaffected

Table 2
Effects of D/RW, grassland type and sampling date on soil microbial nutrients (μg g^{-1} dry soil) and microbial activity measured as soil basal respiration (μl CO_2 hr^{-1} g^{-1} dw soil) in upland grasslands in Littledale, Lancashire, England

Property	Grassland type		D/RW		Sampling date					
	Improved	Unimproved	Control	D/RW	Day 1	Day 3	Day 9	Day 16	Day 30	Day 50
Biomass C	1239.5 ± 82.59^{a}	1870.5 ± 112.34^{b}	1657.4 ± 112.76^{a}	1452.6 ± 111.68^{b}	2091.8 ± 104.6^{a}			799.0 ± 80.88^{b}	1662.6 ± 147.2^{c}	1666.6 ± 94.05^{c}
Biomass N	267.2 ± 33.57^{a}	249.8 ± 20.31^{a}	319.9 ± 34.21^{a}	196.0 ± 14.10^{b}	294.3 ± 28.77^{a}	551.7 ± 65.39^{b}	190.8 ± 16.81^{c}	121.2 ± 10.12^{d}	174.1 ± 17.30^{cd}	200.5 ± 9.94^{c}
Microbial activity	1.2 ± 0.10^{a}	1.0 ± 0.07^{a}	0.9 ± 0.07^{a}	1.4 ± 0.09^{b}	1.5 ± 0.19^{a}	1.6 ± 0.20^{a}	1.1 ± 0.11^{ab}	0.9 ± 0.08^{b}	0.9 ± 0.09^{b}	0.8 ± 0.07^{b}

Values are mean ± SE. Values with the same letters are not significantly different at the $P < 0.05$ level as determined using a Tukey post-hoc test. (Microbial biomass C data are missing for days 3 and 9 due to technical problems.)

by any of the interactions between experimental factors (Table 1).

Microbial community structure, measured on day 30, differed significantly between the soils of the two grasslands, with total PLFA ($F_{1,12} = 46.85$, $P < 0.001$) and total fungal PLFA ($F_{1,10} = 20.34$, $P < 0.01$) being greater in the unimproved than the improved soils (Tables 1 and 3). In contrast, total bacterial PLFA was greater ($F_{1,12} = 136.73$, $P < 0.001$) in the improved than the unimproved soils (Tables 1 and 3). The ratio of fungal-to-bacterial PLFA was also greater ($F_{1,8} = 79.12$, $P < 0.001$; Table 1) in the unimproved compared to the improved soils (Table 3). Total PLFA ($F_{1,12} = 0.12$, $P = 0.7315$) and total bacterial PLFA ($F_{1,12} = 1.79$, $P = 0.2052$) (Table 1) were unaffected by D/RW in both soils (Table 3), but this treatment significantly reduced total fungal PLFA ($F_{1,10} = 5.72$, $P < 0.05$) and the ratio of fungal-to-bacterial PLFA ($F_{1,8} = 5.78$, $P < 0.05$) relative to the controls (Tables 1 and 3).

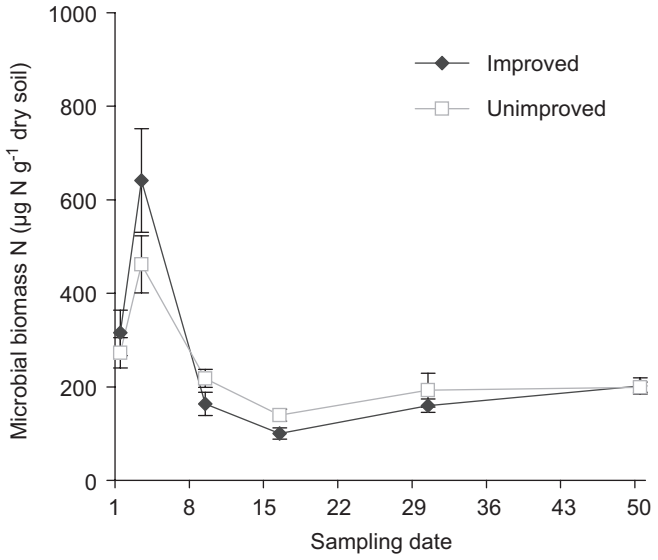

Fig. 3. Effects of the grassland-type treatment on microbial biomass N, over the experimental period. Bars represent means (±SE).

3.2. Soil nutrient leaching

In general, concentrations of DOC, DON and DIN in leachates were increased significantly by D/RW in the improved soil, with no corresponding effects being detected for DOC and DON in unimproved soils. Most of the variance in leachate DOC was attributed to grassland type, ($F_{1,72} = 352.86$, $P < 0.001$) (Table 4), being greater from unimproved than improved soils (Table 5). However, this measure was affected significantly by D/RW, but only in the improved soil ($F_{1,72} = 8.33$, $P < 0.01$) (Fig. 4a) (Table 4). DOC also varied significantly over the experimental period ($F_{5,72} = 13.4$, $P < 0.001$) (Table 4), being greatest on day 1; it then decreased over time (Table 5). For DON, there was a highly significant D/RW × grassland-type interaction ($F_{1,69} = 13.91$, $P < 0.001$), which accounted for 55% of the variance in this measure: D/RW increased leachate DON by 220% in the improved soil, but had no effect in the unimproved soils (Fig. 4b). DON varied significantly across sampling dates ($F_{5,69} = 4.57$, $P < 0.01$) (Table 4), being significantly greater on day 30 than all other sampling dates (Table 5). Leachate DIN was

Table 4
Summary statistics of a two-way ANOVA looking at the effects of D/RW, grassland type and sampling date on the concentrations of leachate DOC, DON and DIN

	Leachate DOC			Leachate DON			Leachate DIN		
	df	F	P	F	P		F	P	
Main effect									
D/RW	1	30.46	<0.001	3.87	ns		28.46	<0.001	
Grassland type	1	352.86	<0.001	0.75	ns		2.37	ns	
Date	5	13.40	<0.001	4.57	<0.01		3.87	<0.01	
Two-way									
Grassland type × D/RW	1	8.33	<0.01	13.91	<0.001		5.18	<0.05	
Date × D/RW	5	0.50	ns	0.74	ns		0.65	ns	
Date × grassland type	5	0.94	ns	0.90	ns		0.42	ns	
Residuals		72		69			69		

Values for leachate DIN were normalised using square-root transformations, values for leachate DOC were normalised using $\log_{10}$ transformations, and values for leachate DON were normalised using reciprocal transformations prior to analysis (ns indicates not significant at $P < 0.05$).

Table 3
Effects of D/RW and grassland type on total PLFA (concentrations in nmol g^{-1} dry soil), total bacterial and total fungal PLFA (measured as a percentage of total PLFA), and on the ratio of total fungal(%)-to-total bacterial (%) PLFA in upland grasslands in Littledale, Lancashire, England

Property	Grassland type		D/RW	
	Improved	Unimproved	Control	D/RW
Total PLFA	395.0±15.23[a]	654.5±40.46[b]	520.0±60.28[a]	529.5±55.14[a]
Total bacterial PLFA	72.7±0.33[a]	66.0±0.79[b]	68.9± 1.38[a]	69.9±1.52[a]
Total fungal PLFA	0.8±0.06[a]	1.2±0.08[b]	1.0±0.10[a]	0.8±0.09[b]
% total fungal:% total bacterial PLFA	0.01±0.001[a]	0.02±0.001[b]	0.02±0.002[a]	0.01±0.001[b]

Values are mean±SE. Values with the same letters are not significantly different at the $P < 0.05$ level as determined using a Tukey post-hoc test.

Table 5
Effects of D/RW, grassland type and sampling date on the concentrations of leachate nutrients (measured as $\mu g\,g^{-1}$ dry soil) in upland grasslands in Littledale, Lancashire, England

Property	Grassland type		D/RW		Sampling date					
	Improved	Unimproved	Control	D/RW	Day 1	Day 3	Day 9	Day 16	Day 30	Day 50
DOC	5.7 ± 0.54^{a}	25.8 ± 2.17^{b}	13.4 ± 1.94^{a}	18.1 ± 2.3^{b}	28.7 ± 6.3^{a}	18.5 ± 3.01^{a}	13.4 ± 2.81^{b}	12.5 ± 2.18^{b}	11.7 ± 2.31^{b}	10.0 ± 2.11^{b}
DON	26.6 ± 5.8^{a}	26.4 ± 4.54^{a}	18.4 ± 2.45^{a}	35.1 ± 6.99^{b}	19.5 ± 2.15^{a}	16.7 ± 1.66^{a}	14.6 ± 2.2^{a}	16.3 ± 1.64^{a}	76.5 ± 16.4^{b}	13.1 ± 0.99^{a}
DIN	28.1 ± 2.64^{a}	23.5 ± 2.53^{a}	17.1 ± 1.56^{a}	35.2 ± 2.82^{b}	16.0 ± 2.54^{a}	17.9 ± 2.79^{ab}	25.3 ± 4.32^{ac}	28.2 ± 4.87^{ac}	33.4 ± 5.21^{bc}	34.1 ± 4.86^{c}

Values are mean $\pm$ SE. Values with the same letters are not significantly different at the $P<0.05$ level as determined using a Tukey post-hoc test.

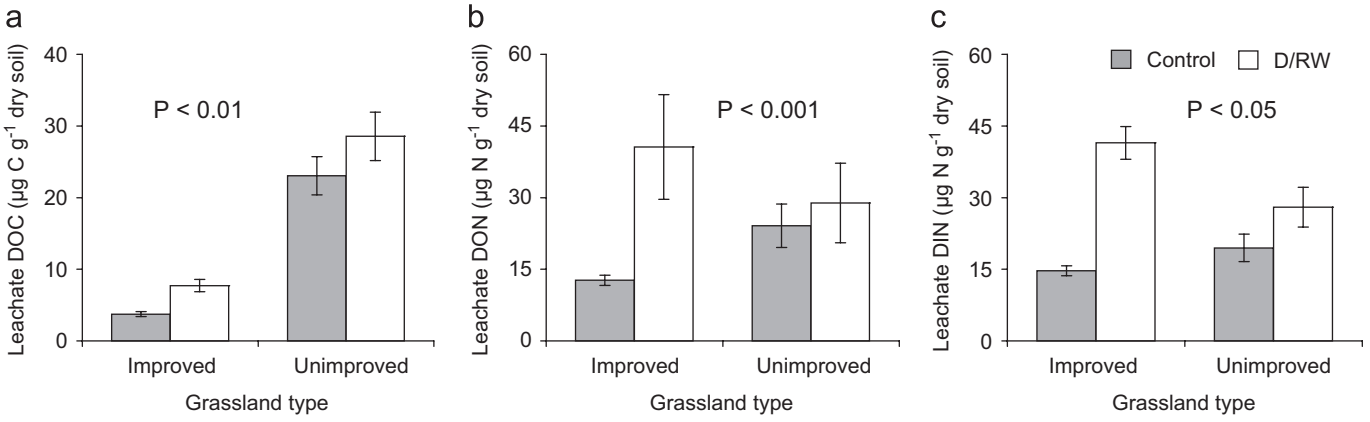

Fig. 4. Effects of the 'drying and rewetting × grassland type' treatment interaction on (a) DOC, (b) DON and (c) DIN concentrations found in leachates. Bars represent means ($\pm$SE).

also significantly affected by the D/RW treatment ($F_{1,69} = 28.46$, $P<0.001$) (Table 4), with concentrations being double that of the control soils (Table 5). As for DON, this effect was especially pronounced in the improved soil (Table 4): D/RW increased leachate DIN by 182% and 44% in the improved and unimproved soil, respectively ($F_{1,69} = 5.18$, $P<0.05$) (Fig. 4c). DIN in leachates varied significantly across sampling days ($F_{5,69} = 3.87$, $P<0.01$) (Table 4) with concentrations increasing over the sampling period (Table 5).

4. Discussion

The aim of this study was to test how soils under different long-term management and with different microbial community compositions differed in their ability to retain nutrients under D/RW stress. Our main finding was that soil of improved and unimproved grassland differed markedly in their susceptibility to D/RW stress, in that nutrient loss was greater from improved than semi-natural unimproved grassland soils. This finding, which is consistent with our main hypothesis, is supported by the finding that D/RW led to a two-fold increase in DOC concentrations, and three-fold increases in DON and DIN concentrations, in soil leachates of the improved soil. In contrast, D/RW had no effect on leachate concentrations of DOC and DON in the unimproved soil, but this treatment did increase DIN in leachates by 44%.

4.1. Microbial responses to drying and rewetting

A combination of mechanisms is likely to have contributed to the increased concentrations of C and N found in leachates after D/RW in both soils, including soil aggregate disruption and the cracking of soil colloids, differing levels of osmotic stress due to differences in soil water potential at equal soil WHC, and microbial processes of death/lysis, osmotic regulation and mineralization. Consistent with several other studies (Bottner, 1985; van Gestel et al., 1993a, b; Grierson et al., 1998; Magid et al., 1999; Wu and Brookes, 2005), we found that D/RW caused a significant reduction in the size of the microbial biomass, measured as biomass C, in both soils. Also, D/RW reduced the abundance of fungal PLFA in both soils, an indicator of fungal biomass (Bardgett et al., 1996) and the ratio of fungal-bacterial PLFA, indicating that fungi are more sensitive to this stress than are bacteria. This is consistent with Hattori (1988), who suggested that microorganisms located in/on the outer part of soil aggregates (i.e. in the larger pores and on aggregate surfaces), where most fungi are found, are more susceptible to D/RW than are microorganisms located within small pores, where bacterial populations dominate. This is also consistent with Griffiths et al. (2003) who found that bacterial communities in soil are resistant to water stress, perhaps due to adaption to moisture variations by regulation of cellular activity.

While the effects of D/RW were consistent in both soils, the reduction in microbial biomass N was especially pronounced in the improved soil, where this measure declined by 46% in response to D/RW. As noted above, this marked decline (46%) in microbial biomass N in the improved soil corresponded with a significant increase in nutrient loss in leachates from this soil. This suggests that D/RW induced increases in DIN and DON concentrations in leachates, particularly from improved soils, are likely to be linked to this decline in microbial biomass N.

Microbial activity, measured as soil CO_2 evolution, increased in both the improved (67% increase) and unimproved (41% increase) soils after D/RW, and was associated with a significant decline in microbial biomass C. Although microbial biomass C declined as a result of D/RW when data were integrated across treatments, the reduction of biomass C was not as pronounced when data were considered for improved and unimproved soils individually. These findings are consistent with Fierer and Schimel (2003) who suggested that the microbial biomass generated a pulse of CO_2 after the rewetting of soil, but without significant microbial cell lysis. They hypothesized that the microbial cells osmoregulated to remove mineralized cytoplasmic solutes to return to equilibrium with their environment after soil rewetting. Similarly, Miller et al. (2005) found little change in the size of the microbial C pool with rewetting relative to the magnitude of CO_2 release. However, they suggested that cumulative CO_2 and DOC release were enhanced in rewetted soils partly through physical disruption that could expose recalcitrant SOM C to leaching and microbial attack; this concurs with many other studies (Powlson and Jenkinson, 1976; van Gestel et al., 1991; Magid et al., 1999; Fierer and Schimel, 2003; Miller et al., 2005; Wu and Brookes, 2005). Interestingly, microbial biomass C and N did appear to respond differently to the drying and rewetting in the two grassland soils, in that microbial biomass N decreased in both the improved and unimproved soils following D/RW, whereas microbial biomass C did not. Further studies are needed to investigate the reason behind these differences and, for example, whether decreases in microbial biomass C were masked due to rapid assimilation of released C by the remaining microbial community, which might explain the apparent lack of effect of D/RW on microbial biomass C.

4.2. Differences in response to soil D/RW between grassland types

There are a number of reasons why the improved and unimproved soils differ in their ability to retain nutrients. First, the differing microbial community compositions of the two grassland types are likely to be an important factor. Analysis of microbial properties show that the soil of the unimproved grassland had a higher microbial biomass and proportion of fungi relative to bacteria in the microbial community than did the improved soil, which is consistent with a number of other studies that show that unimproved grasslands have more fungal-rich microbial communities than their improved counterparts (Bardgett and McAlister, 1999; Bardgett et al., 2001; Donnison et al., 2000; Grayston et al., 2001, 2004; Smith et al., 2003; de Vries et al., 2006). It is likely that these differences in microbial community composition between grasslands will have influenced not only the amount of C and N released into soil solution on cell death/lysis and after osmotic regulation by microbial cells, but also the amount re-immobilized by the microbial community. Indeed, it has been shown that fungi, which are more abundant in the unimproved soil, are especially effective at immobilizing nutrients from soil solution (Bardgett et al., 1993) and that more fungal-rich microbial communities of unimproved grassland soil are effective at sequestering nutrients added to soil solution (Bardgett et al., 2003). Second, increased DIN concentrations in leachates from both grasslands, but especially in the improved grassland, may be related to increased N mineralization after D/RW. The increased availability of organic substrates derived from microbial sources, and also from labile, lighter fractions, of SOM might have stimulated N mineralization (Van Gestel et al., 1993a; Miller et al., 2005). Also, the faster cycling, relatively more bacterial-rich microbial community of the improved soil is likely to have mineralized these organic substrates more quickly than the slower cycling microbial biomass of the unimproved soil, with a greater fungal to bacterial ratio (Wardle et al., 2004), which might have contributed to the greater increase in leachate DIN concentrations from the improved soil after soil D/RW. Third, although both the improved and unimproved soils were subjected to the same soil WHCs, i.e., 55% and 10% WHC, the soil water potentials of the soils differed, being lower in the improved than unimproved soil after drying. This suggests that D/RW caused a greater osmotic stress within improved than the unimproved soil, therefore leading to a greater release of nutrients via microbial cell death/lysis and osmotic regulation by microbial cells. Forth, the improved soil may have been affected to a greater extent by the disturbance of soil aggregates and cracking of soil colloids after the D/RW event and during leaching. The unimproved soil is likely to be less susceptible to this type of disturbance due to its greater organic matter content and the presence of a larger fungal community that would both afford a level of physical protection against the disturbances caused by D/RW stress (Tisdall and Oades, 1982). Cosentino et al. (2006) found that fungal biomass correlated better with aggregate stability than did total microbial biomass, and they suggested that this showed the prominent role of fungi in their contribution to: physical entanglement by the enmeshment of aggregates and particles (Degens, 1997; Tisdall et al., 1997); the production of extracellular polysaccharides which glue particles together (Chenu, 1995; Chenu and Guérif, 1991), and in the production of hydrophobic aliphatic substances (Capriel et al., 1990).

5. Conclusions

We set out to test the hypothesis that differences in soil properties, including shifts in soil microbial community composition, resulting from long-term differences in grassland management influence the ability of soils to retain nutrients under physical stress caused by D/RW. Overall, our data show that nutrient leaching after D/RW was greatest from improved grassland soil, which had a lower microbial biomass and abundance of fungi relative to bacteria than did the unimproved grassland soil. This indicates that the composition of the microbial community in grassland soil can, in part, influence the ability of soils to retain nutrients as a result of rapid dynamic changes in soil moisture status (i.e. localized hydrological pulsing in the rhizosphere), which is common in the spring and autumn in temperate maritime climates (Turner and Haygarth, 2000). Furthermore, changes in microbial community composition, and other soil properties, resulting from land use have implications for soil nutrient leaching potential as a result of hydrological pulsing; fungal-rich soils of low-input, unimproved grasslands are better able to retain nutrients than are their improved counterparts when subjected to hydrological pulsing. More widely, our work demonstrates that soil microbial processes and microbial responses to physiological stress exert a control on soil nutrient release to leachate, and that soil microbes may thus exert an influence on water quality, that is, from an agricultural perspective, largely ignored.

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