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Shifts in the microbial community structure explain the response of soil respiration to land-use change but not to climate warming

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ABSTRACT

Soil stores more carbon (C) than plants and atmosphere combined and it is vulnerable to increased microbial respiration under projected global changes including land-use change and future climate scenarios (mainly elevated temperature). Land-use change is known to have a direct impact on soil organic C and soil respiration (Rs) but the mechanisms that drive these changes remain debatable. Similarly, recent studies and simulation models predict that Rs will respond positively to projected climate warming. However, there are significant uncertainties in the magnitude and mechanisms of this feedback response of Rs to global change. To identify the mechanisms of Rs response to land-use change and climate warming, we first investigated Rs from different land use types. Soil respiration was estimated seasonally from four different Scottish land uses: moorland, birch woodland, grassland and pine forest ($n = 24$). Our results demonstrated that despite a dramatic loss of total C and nitrogen (N) in the soils under birch trees, the Rs in the birch woodland was similar to that of the moorland and pine forest, with Rs in the grassland being significantly higher. The microbial community structure, estimated by Multiplex Terminal-Restriction Fragment Length Polymorphism (MT-RFLP) and 454 pyrosequencing, was significantly different under each land use type. A strong correlation of Rs with soil properties (pH, inorganic N, C:N ratio and moisture) and with microbial community structure was identified.

To test the impact of elevated temperature on Rs and to identify potential microbial mechanisms, we performed laboratory incubation studies. Soils from different land uses were incubated at 7 °C (mean annual temperature (MAT) in Scotland) and 10 °C (MAT + 3 °C) with and without the presence of a labile (^{13}C-glucose) and recalcitrant (^{13}C-lignin) form of C to identify the active groups of microbes and to determine the role of substrate availability on feedback response. The warming treatment induced an increase in Rs rates in all soils. The magnitude of the Rs response to warming was modulated by the land use types, and the Rs was more prominent in soils with high C contents. The addition of glucose substantially increased both total and rate of Rs compared to no substrate- and lignin-amended soils, providing evidence of labile C depletion as a mechanism for the thermal response of Rs. The warming treatment did not impact the composition of the active or total microbial community as revealed by phospholipid fatty acid-stable isotope probing (PLFA-SIP), MT-RFLP and 454 pyrosequencing. Our results showed that the microbial metabolic activity was higher under warming treatment suggesting that a positive feedback of Rs to increased temperature is mediated by changes in substrate availability and microbial metabolic rates.

1. Introduction

Soil stores 2500 Pg of carbon (C) which is significantly more than the C locked in the plant and atmospheric pools (Singh et al., 2010). Soil microbial respiration (Rs) emits ~60 Pg annually from

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the soil organic carbon (SOC) pool, which is compensated by plant photosynthetic activities (Bradford, 2013). This balance is vulnerable to global change, including land-use change and climate warming, resulting in higher rates of Rs thereby increasing the atmospheric concentration of CO_2. Currently, there is a strong global trend towards the afforestation of marginal land, driven by a high demand for timber and the potential for forests to sequester atmospheric CO_2. Land-use change can potentially have a significant impact on Rs through its influence on microclimate, soil abiotic and biotic properties. For example, previous studies found low microbial biomass and Rs following conversion of pasture into forest (Ross et al., 2002; Scott et al., 2006). However, the direction, magnitude and mechanisms of Rs response to land-use change remain controversial (Barger et al., 2011; Zhang et al., 2013). Land-use change can have a significant direct impact on soil microbial communities, which are the main components of Rs, *via* a transfer and shift in quality and quantity of photosynthates and litter. Indirectly, land-use change can impact microbial communities *via* a shift in soil abiotic properties such as moisture, porosity and nutrient availability (Macdonald et al., 2009). However, we have little mechanistic knowledge of the response of the various components of microbial community (biomass, structure and diversity) to land-use change, and the consequences for Rs. Identifying the key microbial mechanisms is important for understanding the specific effect of land-use change on Rs.

Current soil carbon and Earth-system models estimate a positive and exponential feedback loop of Rs in response to global warming (Friedlingstein et al., 2006). This assumption is supported by short-term experiments which have demonstrated an exponential relationship between Rs and increasing temperature (Davidson and Janssens, 2006). However, there are significant uncertainties regarding the feedback response and it is proposed that, like plant and animal metabolisms, microbial respiration can acclimatise to a global increase in temperature despite a current debate in the literature (Hartley et al., 2007; Brabford et al., 2008; Bradford, 2013; Karhu et al., 2014; Wei et al., 2014). For example, Bradford et al. (2008, 2010) reported acclimation of soil respiration even in the presence of sufficient substrate availability, suggesting that the microbial community was able to adjust its metabolic activities to increasing temperature. However, other studies reported no acclimation of Rs (Hartley et al., 2007, 2008; Vicca et al., 2009) and argued that the increasing Rs response to temperature was linked to substrate depletion. Recent studies also described a potential role of shifts in the soil microbial biomass or community structure in Rs thermal response (Thiessen et al., 2013; Wei et al., 2014). Such contrasting observations preclude the development of a general explanatory mechanism for Rs response to climate warming. Understanding both magnitude and mechanisms is important to reduce the uncertainties in the predicted 40% increase in Rs feedback response associated with climate warming (Stocker et al., 2013).

A recent study conducted across the globe concluded that soils with high C contents and from cooler parts of the world are more vulnerable to C loss under global warming (Karhu et al., 2014). The authors also reported that rather than acclimation, most soils showed an enhanced response to climate warming. This was more pronounced in temperate and boreal ecosystems which store more than 50% of terrestrial C. However, there are still significant knowledge gaps on the mechanisms of Rs response to predicted global warming. Several mechanisms that can determine the temperature sensitivity of Rs have been proposed, including: (1) loss of labile C (substrate depletion theory), (2) change in microbial physiology, (3) change in microbial community composition, or (4) decrease in carbon-use efficiency in the microbial community. In modelling studies, it was suggested that Rs acclimation can be explained by a single factor of substrate depletion (Kirschbaum, 2004; Knorr et al., 2005). One aspect that received less attention is substrate origin. Because soil C is a direct product of the decomposition of plant debris, it is expected that different land uses will result in soil C compounds which differ in their structure and composition (C quality). Invariably, microbial community structure, which is a key regulator of Rs, is altered under land-use change (Costa et al., 2006; Macdonald et al., 2009). Because two key components of the Rs response to temperature are proposed to be substrate quality and microbial community composition/biomass (Hartley et al., 2007; Bradford et al., 2008; Singh et al., 2010), it can be predicted that Rs from different land uses will respond differently to climate warming.

Our aim was to investigate the mechanism(s) that can best explain the Rs response to land-use change and climate warming under carbon-rich ecosystems. To test this, we first investigated the impact of land-use change on Rs and hypothesised that changes in the microbial community structure will explain the Rs response to afforestation. We then carried out incubation studies in order to identify the mechanism(s) which could explain the temperature sensitivity of Rs in the presence and absence of additional labile (glucose) or recalcitrant (lignin) substrates. We hypothesised that both substrate availability and microbial community (biomass, structure and/or physiology) response will be linked to the temperature sensitivity of the soil C. We examined the temperature sensitivity of Rs from different land uses to address key questions: whether Rs acclimatises and which mechanism(s) (substrate availability and/or microbial acclimation) best explains Rs response to climate warming.

2. Material and methods

2.1. Field sites and soil sampling

The soils and sites used in this experiment were part of a bigger seasonal project and a detailed description can be found elsewhere (Nazaries et al., 2013). In brief, three sites were selected (Craggan, Glensaugh and Tulchan, Scotland). The land-use changes investigated were (i) moorland colonisation/afforestation by (old) birch woodland (Craggan and Tulchan) and (ii) afforestation of grassland with pine trees (Glensaugh). Soil samples (12 replicates per land use) were carefully excavated using 10-cm wide stainless steel rings over a 0–10 cm profile. The soil cores were kept intact inside the sampling rings, wrapped in cling film and brought back to the laboratory, where they were placed in a controlled-environment room (70% humidity, no light) and incubated for 24 h (without the cling film) at a temperature close to the site's air temperature at the time of sampling: 5 °C in winter, 10 °C in spring, 15 °C in summer and 20 °C in autumn (see Nazaries et al. (2013) for more details). Headspace CO_2 concentration (considered soil respiration − Rs) and microbial communities were measured seasonally. The chemical soil properties were measured in autumn 2008 and summer 2009 (pH, total C and N, NH_4^+ and NO_3^- content and soil moisture) while the physical soil properties were only measured in summer 2009. For the temperature sensitivity incubation of the present study, only the soils sampled in summer 2009 were used.

2.2. Soil respiration (Rs) measurements

Headspace gas samples were taken using closed-bottom PVC chambers (~9 L) fitted with a gas sampling tube and a 3-way tap. Out of the 12 replicates from each land use, three soil cores per chamber were used, with four chambers for each land use ($n = 4$). Two supplementary chambers containing no soil cores were also used in order to detect the level of “background flux”, in other

words the flux value for which no real flux was measured. Immediately after locking the lid of the chambers (T_0), 50 mL of air was sampled from the chamber's headspace using a plastic syringe fitted with Luer lock and 3-way tap, and quickly injected into a pre-evacuated container. Headspace sampling was repeated after 30, 60 and 90 min (T_{30}, T_{60} and T_{90}, respectively). Gas samples of CO_2 were quantified by gas chromatography (GC model: Hewlett Packard 5890, Series II, Avondale, PA, USA) (Mapanda et al., 2010). Carbon dioxide gas fluxes were estimated as the change in concentration inside the headspace over the incubation time using the linear (Matthias et al., 1980) and quadratic (Wagner et al., 1997) models. The linear model tends to underestimate the real fluxes, but the quadratic model is less sensitive, so a "best-fit" approach was favoured to choose the model that best fitted the nature of the data points of a particular time series (De Klein and Harvey, 2012). Once the flux measurements were made, the soils were sieved through a 2-mm mesh and stored at 4 °C. Physical and chemical analyses were performed on all soils using standard methods (Nazaries et al., 2013).

2.3. Determination of the microbial community structure by multiplex T-RFLP (MT-RFLP) and 454 pyrosequencing

To characterise the microbial community a polyphasic approach was implemented in order to examine the response of microbial communities at different resolutions. We used MT-RFLP analysis to characterise bacterial, fungal and archaeal community structure, as described by Singh et al. (2006). Samples were split into two sets: the first set constituted of the original soils from the (summer) field collection (n = 8 for moorland and n = 8 for birch woodland from two sites (Craggan and Tulchan); n = 4 for grassland and n = 4 for pine forest from the Glensaugh site). The second set represented the soil samples following the temperature incubation experiment, that is, the soils incubated with water, ^{13}C-lignin and ^{13}C-glucose at MAT and MAT + 3 °C (see below). For each set, the soil was analysed for the community structure of Bacteria and Archaea (16S rRNA gene) and Fungi (ITS) (Singh et al., 2006).

We then used 454 pyrosequencing to determine bacterial diversity, community composition and phylogenetic distribution using methods described in Singh et al. (2014). Briefly, the pyrosequencing of 16S rRNA genes was performed on a Roche Junior Titanium Series (Roche, USA). Using the modified primers PRK341F (5′-CCTAYGGGRBGCASCAG-3′) and PRK806R (5′-GGACTACNNGGGTATCTAAT-3′) (Caporaso et al., 2010; Xu et al., 2012), a 466-bp fragment of 16S rRNA gene was amplified. We used the Quantitative Insights Into Microbial Ecology (QIIME version 1.6.0) software package (Caporaso et al., 2010) to analyse data including assessment of the main bacterial phyla abundance. Barcode, linker primer and reverse primer sequences were removed from the raw sequence reads using the 'split_libraries.py' script while setting a minimum sequence length of 200 and a minimum quality score of 20. We then used the 'Acacia' tool with default options to remove pyrosequencing noise (Bragg et al., 2012). Potential chimeras were removed using the UCHIME chimera detection utility of the USEARCH (version 6.0.307) tool (Edgar et al., 2011). Similar sequences were binned into OTUs using the 'UCLUST' method (minimum pairwise identity of 97%). For the first set of samples (before incubation), three replicates were analysed by pyrosequencing using published methods (Singh et al., 2014) in order to determine the microbial diversity and composition of the soil samples. In order to test whether the incubation at MAT + 3 °C had an impact on the microbial community at the phylogenetic level, we repeated the 454 pyrosequencing analysis along with MT-RFLP. However, for 454 pyrosequencing of the second set of samples (after incubation), the PCR products of replicates were pooled. Also, only the soils incubated with water and ^{13}C-glucose were analysed based on respiration and MT-RFLP data (see Results section).

2.4. Response of soil respiration to climate warming as modulated by the availability and type of substrate

Our approach to test the response of Rs to warming treatment was similar to that of Tucker et al. (2013). Laboratory incubation studies were carried out in three sequential phases: a stabilisation phase (Day 1–14), an acclimation phase (Day 15–25) and a response phase (Day 26–27). In the stabilisation phase, the soils were sieved using a 2-mm sieve and a subsample of each was weighed into a plastic bag before being placed inside an incubator at 7 °C, which represented the mean annual temperature (MAT) in Scotland for the period August 2008 to July 2009 (www.metoffice.gov.uk), for a two-week conditioning period prior to the start of the experiment. This was done to minimise the impact of sieving on both labile C availability and microbial communities (Karhu et al., 2014). Aerobic conditions were maintained by placing a paper towel in the neck of the bag (out of contact with the soil), and this was then sealed with an elastic band. The bags were kept in a plastic box with a dish of soda lime to absorb the carbon dioxide produced, and a dish of water to prevent desiccation. For the acclimation phase, the soils were weighed into two sets of 96-well plates (~0.4 g per well). One set was incubated at 7 °C (MAT in Scotland, as indicated above) and the other set at 10 °C, which represented the MAT + 3 °C or the temperature increase predicted by the Intergovernmental Panel on Climate Change (IPCC) (Stocker et al., 2013), to allow acclimation and utilisation of labile C from the samples. The soil moisture was adjusted to 60% of the soil water holding capacity as described by Campbell et al. (2003). Our data suggested that Rs was stabilised during the first two phases. The response phase was carried out for 48 h during which Rs was measured at regular intervals (4 h, 24 h and 48 h). Because most of the responses could be captured after 24 h of incubation (see Supplementary Fig. 1A), we focused our analysis on the 24-h data in order to make it comparable to previous studies (Bradford et al., 2008; Tucker et al., 2013).

To investigate the impact of substrate availability and quality, we measured Rs and mass-specific respiration (Rmass; see Bradford et al. (2008)) response in the presence of two C sources along with basal Rs using the MicroResp™ technique (Campbell et al., 2003). Two C substrates (D-glucose and lignin, from Sigma–Aldrich) were applied (25 μL of 30 mg per gram of fresh soil) to four technical replicates so as to account for experimental variation. Another set of replicates received 25 μL of distilled water (dH_20) and represented the basal respiration (no C source). Each set of plates was then incubated at 7 °C (MAT) or at 10 °C (MAT + 3 °C). Rs was measured 4, 24 and 48 h after the start of the experiment. This short-term incubation after acclimatisation was carried out to align our work with current simulation modelling which include respiration measurements after the first 24 h, both for plant and microbial response to climate warming (Atkin and Tjoelker, 2003; Kirschbaum, 2006; Bradford et al., 2008; Tucker et al., 2013).

2.5. Identifying the active microbial communities involved in carbon decomposition under climate warming

Microbial communities that were actively involved in the Rs response were identified by stable-isotope probing coupled with phospholipid fatty acid extraction (PLFA-SIP) analysis using an approach of Thornton et al. (2011). We used this approach because PLFAs are good biomarkers for estimating microbial biomass. When coupled with stable-isotope probing (SIP) using labelled substrates, PLFAs can provide useful information on the microbes that actively

use these substrates (Boschker et al., 1998; Singh et al., 2009). Separately, 5 g of soil were weighed into a 125-mL Wheaton bottle (Sigma–Aldrich). Parafilm was placed over the neck of the bottle, held in place with an elastic band and pierced to maintain aerobic conditions. This was carried out in duplicate for each field replicate, and for each incubation temperature – a total of 4 bottles per field replicate. Samples without substrate amendment were incubated as controls. One set of 18 bottles representing each site was incubated for 10 days at 7 °C and the other set of 18 bottles at 10 °C, as above. Then, the substrates were added at the rate of 240 μg of ^{13}C-glucose or ^{13}C-lignin per 5 g of fresh soil (Waldrop and Harden, 2008). 14.4 mg of ^{13}C-glucose was dissolved in 30 mL of dH_2O, and 500 μL of this solution was added to two sets of 8 soils for each site. The same concentration of ^{13}C-lignin was added to each set of 8 soils per site. The control samples each received 500 μL of dH_20. All bottles were sealed with a grey butyl rubber septum held in place by a crimped aluminium cap (Sigma–Aldrich) and the samples were returned to their respective temperature for 5 days for measurements of Rs and active microbial communities. The soils were then stored at −20 °C before freeze-drying under vacuum at −50 °C and subsequent milling before PLFA-SIP analysis.

2.6. Statistical analysis

Many variables were $\log_{10}$-transformed for statistical analysis in order to obtain a normal distribution. These were the MicroResp™ data (cumulated percentages of CO_2 production and CO_2 production rates), the mass-specific respiration (Rmass), the PLFA and pyrosequencing (phylum and class levels) datasets and the net CO_2 flux data as well as some environmental variables (total carbon and nitrogen, and ammonium content and bulk density). Repeated measurements linear mixed model (repeated LMM) was applied to the MicroResp™ dataset in order to estimate the effect of land-use change and temperature on soil respiration over the incubation period (nested design) and with each carbon substrate. The incubation time and the soil replicates were used as random effects with a model of first order antedependance.

Information theory and multi-model inference based on the Akaike's Information Criterion (AIC) (Burnham and Anderson, 2002; Symonds and Moussalli, 2011) was applied to Rs in order to investigate which soil abiotic and biotic properties could explain the variation observed in Rs. This approach relies on ordinary least-squares (OLS) linear regression analysis and is considered better than multiple linear regression as it does not rely on null hypothesis testing (Burnham et al., 2011; Mundry, 2011). Instead of performing a stepwise selection of the predictor variables of interest (like with a classical multiple linear regression), this approach uses an 'all subset' analysis during which all possible models are ranked based on their AIC value, with the lowest AIC corresponding to the 'best approximating model' (Burnham and Anderson, 2004; Burnham et al., 2011; Martins et al., 2015).

The PLFA data (% of total abundance on a molar basis) were analysed by principal component analysis (PCA) including only the fatty acids which abundance overall samples was more than 1% of the total PLFA abundance. Out of the 48 PLFAs initially detected, 23 were retained representing approximately 94% of the total abundance. A scree plot analysis was run on the principal component (PC) scores produced and the first six PCs (out of 23) were deemed to significantly contribute to the total effect, accounting for 90% of the total variance. The PC scores were further subjected to canonical variates analysis (CVA) in order to discriminate between the treatments. MT-RFLP and 454 pyrosequencing data were analysed by PCA and principal coordinate analysis (PCoA), respectively.

A rarefaction analysis of the pyrosequencing data indicated that further analysis would be based on 9000 sequences per sample. The beta diversity data were subjected to PCoA using a weighed UniFrac distance matrix (Lozupone and Knight, 2005; Lozupone et al., 2011). Furthermore, treatment effect was tested by ANOVA on the phyla and classes that showed a relative abundance of more than 0.5% the total abundance.

The significance of the effect of land use, carbon source and temperature on the different variables (soil properties and Rs, induced respiration and PLFA data, as well as ordination scores from CVA, PCoA and PCA) was estimated by a nested linear mixed model (LMM) approach (*i.e.* residual maximum likelihood – REML). Mean separation was investigated using the Bonferroni correction as post-*hoc* test. Throughout the manuscript, each land use of each of the three sites has four replicates. Thus, for Grassland and Pine (Glensaugh site), $n = 4$ whereas for Moorland and Old Birch (Craggan and Tulchan sites), $n = 8$. However, for the pyrosequencing analysis from the field collection, triplicates were used (see above) and as a consequence $n = 3$ for Grassland and Pine and $n = 6$ for Moorland and Old Birch. All statistical analyses were performed using GenStat® 16th edition (VSNI, UK). Model selection and multi-model inference were assessed with SAM (Spatial Analysis in Macroecology, version 4) (Rangel et al., 2010).

3. Results

3.1. Land-use change impact on soil respiration and microbial community structure

The four land use types displayed differences in their abiotic properties (Table 1), except for water-filled pore space (WFPS, $P = 0.129$). Afforestation did not consistently impact all soil characteristics. The change from grassland to pine forest did not alter the soil moisture or structure (bulk density, porosity and particle size), whereas afforestation significantly decreased soil pH ($P < 0.001$) and increased nitrate (NO_3^-) content ($P < 0.001$). On the other hand, moorland afforestation by birch woodland significantly affected the soil chemistry due to a dramatic loss of total C ($P < 0.001$) and NO_3^- content ($P < 0.001$) as well as a decrease in soil moisture and a change in soil structure (Table 1). At the seasonal level, the NH_4^+ and NO_3^- contents varied significantly ($P = 0.016$ and $P < 0.001$, respectively; see Table 1) but no seasonal or habitat-related trend could be observed. An effect of afforestation was detected with Rs being significantly lower in the soils under pine forest compared to grassland (Fig. 1, $P < 0.001$). There was a non-significant trend towards increased Rs in the old birch woodland soils compared to the soils from the moorland sites. Rs varied seasonally ($P < 0.001$), being lowest in winter (Fig. 1).

MT-RFLP data showed minor but statistically significant seasonal variations in microbial community structure. For example, the fungal community was slightly different in autumn and winter in the soils under pine forest. Similarly, the archaeal community structure was different in the pine forest during summer. However, there was no significant seasonal shift in the bacterial community (data not shown). Principal component analysis of fungal, bacterial and archaeal communities showed that the soil microbial structure differed between the different land uses. MT-RFLP revealed that the fungal community was affected by afforestation in the moorland (Fig. 2A; $P < 0.001$). The fungal community under birch forest was significantly different from all other land-uses. A similar effect was detected at the bacterial and archaeal community level with the afforestation of grassland (Fig. 2B and C; $P = 0.001$ and $P < 0.001$, respectively). The difference in the bacterial community was stronger when 454 sequencing data were used, which was expected given the higher resolution of next-generation sequencing. The weighed UniFrac distance method demonstrated a strong

Table 1
The effect of land-use change and season on some soil properties. For each variable, the data represent means (n = 8 for Moorland and Old Birch; n = 4 for Grassland and Pine) from the summer 2009 sampling. The letters (a, b, c) show the treatments with significant difference (P values in bold) according to pairwise comparison following the Bonferroni procedure as post-hoc test ($\alpha < 0.05$). The P values obtained following nested LMM analysis are also presented to indicate any seasonal effect. N/A means non-applicable as soil structure properties (particle size, bulk density and derived calculations) were only measured in summer. The standard deviations (SD) are presented in the brackets for each variable. When two values are shown, they represent the lower and upper limits of the SD of a $\log_{10}$-transformed dataset due to the loss of symmetry following back-transformation.

Land use	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N ratio	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	Moisture (% weight)	Particle size (%)			WFPS (%)	Bulk density (g cm^{-3})	Porosity (%)
								Clay	Silt	Sand			
Moorland	3.46^a	81.41^c	3.09^b	26.63^c	96.38^b	115.9^b	83.72^c	1.17^a	15.70^a	83.14^b	62.14^a	0.30^a	88.43^c
	(0.12)	(11.84, 13.60)	(0.52, 0.54)	(3.20)	(40.4, 69.4)	(25.6)	(5.30)	(0.79)	(6.89)	(7.65)	(4.74)	(0.088, 0.095)	(3.61)
Old Birch	3.51^a	32.86^b	1.99^{ab}	22.00^b	95.50^b	38.9^a	56.56^b	3.01^b	28.52^b	68.48^a	67.78^a	0.82^b	66.63^b
	(0.15)	(24.01, 54.57)	(1.56, 1.79)	(4.14)	(52.1, 114.4)	(31.1)	(17.49)	(1.08)	(11.28)	(12.05)	(8.58)	(0.455, 0.607)	(21.43)
Grassland	4.21^c	3.84^a	0.40^a	9.72^a	37.33^{ab}	119.6^b	27.54^a	3.82^{bc}	33.62^b	62.56^a	71.79^a	1.39^b	47.53^{ab}
	(0.19)	(0.415, 0.427)	(0.055, 0.056)	(0.66)	(7.14, 8.83)	(86.9)	(2.75)	(0.76)	(2.54)	(3.11)	(10.37)	(0.106, 0.111)	(4.04)
Pine	3.92^b	3.37^a	0.30^a	11.39^a	18.03^a	209.9^c	29.91^a	4.59^c	39.15^b	56.26^a	58.65^a	1.56^b	40.81^a
	(0.11)	(0.171, 0.173)	(0.016, 0.016)	(0.08)	(0.93, 0.98)	(20.5)	(3.23)	(0.62)	(2.59)	(3.16)	(4.48)	(0.204, 0.222)	(8.11)
F_{prob} (Land use)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	N/A	N/A	N/A	0.129	**<0.001**	**<0.001**
F_{prob} (Land use/Season)	0.695	0.496	0.604	0.183	**0.016**	**<0.001**	0.904	N/A	N/A	N/A	N/A	N/A	N/A

Abbreviations: pH = potential hydrogen (negative log of the activity of the hydrogen ion in an aqueous solution); C = carbon atom; N = nitrogen atom; C:N = ratio of total C-to-total N; NH_4^+ = ammonium ion; NO_3^- = nitrate ion; WFPS = water-filled pore space.

Fig. 1. The effect of season on soil respiration (Rs). Data show the net CO_2 fluxes (error bars represent standard deviations) at the different seasons (n = 4) for all the different land uses investigated. For flux estimation, a "best-fit" approach was favoured in order to choose the most suitable model (linear *vs.* non-linear regression). It was based on the behaviour of the second derivative of the quadratic regression: if its value was negative while the apparent linear flux was positive, then the quadratic model was chosen. Inversely, when the second derivative displayed a positive value while the apparent linear flux was positive, the linear flux was favoured. On average, 71% of the fluxes were estimated using the linear model.

impact of afforestation on microbial communities for both moorland and grassland (Fig. 2D, P < 0.001).

Further analysis of the pyrosequencing data (normalised to 9000 sequences/sample) revealed site differences in terms of relative abundance at the phylum/class levels. The Moorland/Old Birch sites (Craggan and Tulchan) were characterised by a higher abundance of the phyla Acidobacteria (classes Acidobacteria and Solibacteres) and Planctomycetes (Phycisphaerae class) (Fig. 3 and Supplementary Tables 2 and 3). On the other hand, the Grassland/Pine site (Glensaugh) revealed a significantly higher abundance of microbes of the phyla Proteobacteria (P < 0.001), Gemmatimonadetes (P < 0.001), Chloroflexi (P < 0.001) and Firmicutes (P < 0.001) (Fig. 3 and Supplementary Table 2). Although Actinobacteria were the most abundant phylum in our soils, there was not any land use and afforestation effect (P = 0.153; Supplementary Table 2). Similarly there were differences in relative abundance at the class, order and genus levels (Fig. 3 and Supplementary Table 2). Additionally, the afforestation of moorland had an impact on specific microbial classes. In particular, the class Acidobacteria-2 was significantly more abundant in the birch woodland and pine forest (P < 0.001, Supplementary Table 2), whereas moorland afforestation decreased the proportion of microbes of the phylum Verrucomicrobia (P < 0.001, Fig. 3), especially the Pedosphaerae class (P = 0.008, Supplementary Table 2), as well as the class Acidimicrobiia (Actinobacteria) (P = 0.021, Supplementary Table 2). Similarly, afforestation of grassland induced a statistically significant loss of Deltaproteobacteria (Proteobacteria) and Spartobacteria (Verrucomicrobia) (P < 0.001, Supplementary Table 2). There was no effect of land use on the various diversity indices tested (PD whole tree, Chao1, richness, Shannon) based on 9000 sequences per sample (data not shown).

The seasonal abiotic and biotic soil properties were subjected to multiple regression analysis against Rs in order to identify which factors could influence Rs. Following the approach of information theory and multi-model inference based on AIC, there were 9 models with a low ΔAIC_c (value less than 2; cumulated AIC_c w_i ~39%) which were potentially as good as the 'best model' (*i.e.* the model with the lowest AIC_c). When the bacterial PC1 was discarded from the 'best' model, the precision of the model

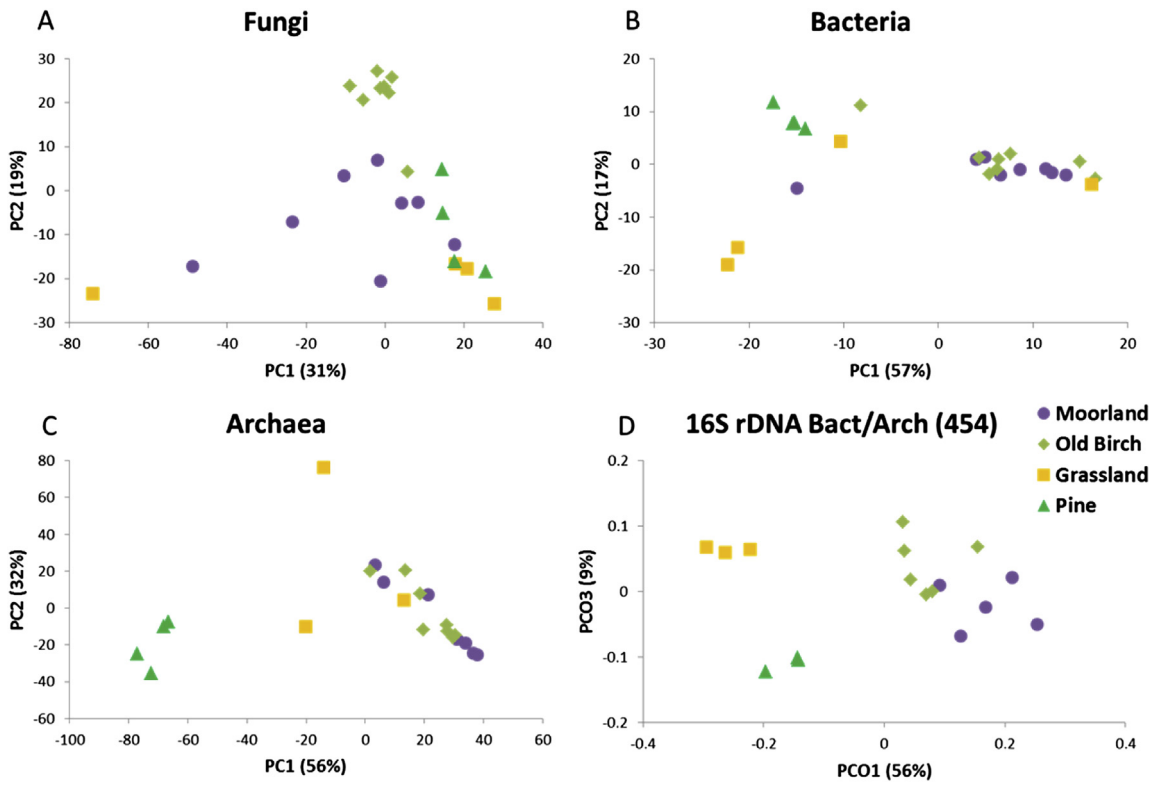

Fig. 2. The effect of land use and land-use change on the microbial community structure. For each microbial group (Fungi (A), Bacteria (B) and Archaea (C)), data represent the principal component (PC) scores of each soil sample (n = 8 for Moorland and Old Birch; n = 4 for Grassland and Pine) obtained from the MT-RFLP. The significance tests are presented in Supplementary Table 1. In quadrat (D), data represent the scores from the principal coordinate analysis (PCoA) of the β diversity. For each land use of each site, n = 3 except for the Old Birch in Tulchan (one replicate was omitted from the analysis due to extremely low number of reads from pyrosequencing).

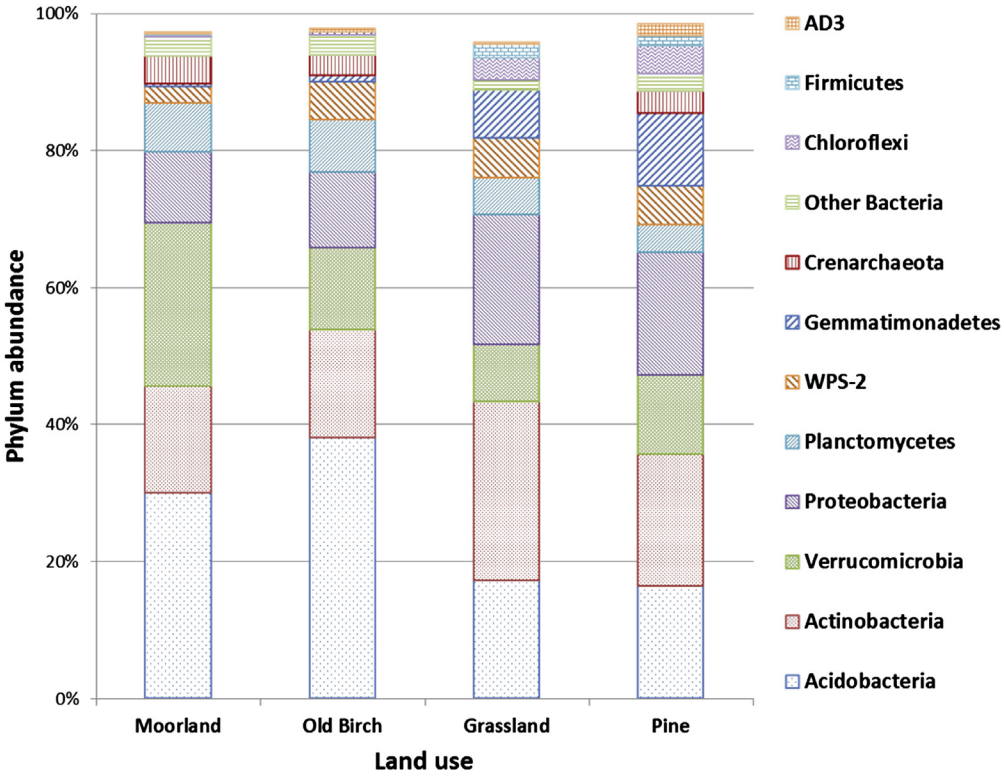

Fig. 3. Most abundant phyla of microorganisms detected in the soils under different land uses. Data represent the mean percentage presence of a particular phylum (n = 6 for Moorland and Old Birch; n = 3 for Grassland and Pine). Only phyla that constituted >0.5% of the total phylum abundance were included in the analysis, representing ~98% of phylum presence in all samples. More details on the statistical differences of the effect of land-use change on the different phyla and classes detected are provided in Supplementary Tables 2 and 3.

Table 2
Predictor variable 'importance' in explaining Rs variation across the different land uses and seasons (n = 91). A variable importance value represent the sum of the Akaike weights (AIC_c w_i) of all the models that included the predictor of interest. Also presented are the parameter estimates averaged across the 1023 OLS regression models (this is called model averaging). The average explanatory power values were: $R^2 = 0.459$, $R^2_{adj} = 0.406$, $AIC_c = 4.269$ and $\Delta AIC_c = 5.015$. Variables are coloured according to the type of class they represent: orange for abiotic properties, kaki for the bacterial community structure (T-RFLP data), grey for *Fungi* and blue for *Archaea*.

	Constant	pH	C:N ratio	Moisture	NH_4^+	NO_3^-	Bacteria		Fungi		Archaea
							PC1	PC2	PC1	PC2	PC2
Variable importance (Rank)	-	1.000 (#1)	0.937 (#3)	0.744 (#5)	0.984 (#2)	0.719 (#6)	0.906 (#4)	0.441 (#9)	0.442 (#8)	0.272 (#10)	0.457 (#7)
Coefficient	-2.762	0.899	0.024	-0.005	0.342	-0.123	-0.005	0.005	0.002	0.001	-0.001
Std Coeff.	0.000	0.930	0.576	-0.447	0.427	-0.202	-0.260	0.228	0.174	0.063	-0.167
Std Error	0.790	0.183	0.008	0.002	0.107	0.044	0.002	0.002	<.001	<.001	<0.001
95% Lower	-4.311	0.540	0.007	-0.009	0.133	-0.210	-0.008	0.002	<0.001	<0.001	-0.002
95% Upper	-1.214	1.259	0.040	-0.002	0.552	-0.037	-0.001	0.009	0.004	0.002	<0.001

dropped from 43% to below 39% and the resulting model was almost seven times less likely to explain changes in Rs (ER = 6.6 – see Supplementary Table 4).

Table 2 summarises the 'importance' of each of the ten selected predictor variables relative to all 1023 OLS regression models. The six most 'important' predictors were those included in the 'best' model (Supplementary Table 4), with soil pH being the most important of the variables (AIC_c $w_i = 1$ and highest standardised regression coefficient of 0.93) to predict Rs variation and was closely followed by NH_4^+ content, C:N ratio and *Bacteria* PC1 (Table 2). *Fungi* PC2 scores seemed to be the least important predictor variable (AIC_c $w_i = 0.272$). Furthermore, we identified that Rs was negatively influenced by the soil moisture and NO_3^- content, but positively linked with soil pH, NH_4^+ content and C:N ratio. Bacterial, fungal and archaeal community structure were also strongly correlated with Rs (Table 2). Total C content could not be included in the multi-model inference approach due to a high level of collinearity with other variables. Further analysis indicated that a simple linear regression between Rs and total C content was only significant when land use was included in the model ($P < 0.001$, $R^2_{adj} = 0.74$) while total C was not correlated with the changes in Rs ($P = 0.232$) (data not shown).

3.2. Response of soil respiration to climate warming as modulated by availability and type of substrate

Rs at different temperatures (MAT: 7 °C and MAT + 3 °C: 10 °C) was tested for basal respiration as well as in the presence of two different carbon sources: D-glucose and lignin. Rs was significantly increased over the duration of the incubation (48 h) with basal respiration being the lowest and glucose addition displaying the highest values ($P = 0.003$) (Supplementary Fig. 1 and Supplementary Table 5). There was a significant increase in total microbial biomass in soils amended with glucose and lignin (Supplementary Fig. 3). Furthermore, the cumulated CO_2 production during the incubation at MAT was unchanged for basal and lignin-induced respiration whereas they significantly increased in the presence of glucose at MAT + 3 °C ($P < 0.001$, Supplementary Table 5). A land-use effect was observed with soils under moorland showing the highest Rs rates irrespective of the C source (Table 3). Over the duration of the incubation, there was a consistent effect of temperature on Rs which was stronger and statistically significant in soils amended with glucose ($P = 0.048$, Table 3 and Supplementary Fig. 1). However, the temperature effect on Rs was stronger after 24 h of incubation for both basal respiration ($P = 0.007$) and glucose-amended respiration ($P < 0.001$) which displayed statistically significant increased Rs under elevated temperature but in the moorland soils only (Supplementary Fig. 2 B1 and B2, respectively). The response to warming treatment in soil with lignin amendment was also consistently positive but statistically insignificant (Supplementary Fig. 2 B3). Overall, the Rs was stabilised after 24 h of incubation, except in soils amended with glucose (Supplementary Fig. 1). We then calculated the mass-specific respiration (or Rmass) for each sample (Fig. 4). Our data showed a moderate but consistent increase in Rmass in most of the

Table 3
The effect of land-use change and temperature on Rs (CO_2 production rate) with and without labile or recalcitrant forms of carbon. Data represent the predicted means (n = 8 for Moorland and Old Birch; n = 4 for Grassland and Pine) of the CO_2 production rate (μg CO_2–C g^{-1} h^{-1}) and P values obtained from repeated measurements LMM analysis. The letters (a through d) show the treatments with significant difference (P values in bold) according to pairwise comparison following the Bonferroni procedure as post-hoc test ($\alpha < 0.05$). The effect of land use alone is not shown here but was always highly significant ($P < 0.001$) irrespective of the carbon source.

	Basal respiration		Glucose-induced respiration		Lignin-induced respiration	
	7 °C	10 °C	7 °C	10 °C	7 °C	10 °C
Moorland	1.72^b	1.94^b	3.83^c	5.89^d	3.24^b	3.51^b
Old birch	0.62^a	0.70^a	1.97^{abc}	3.06^{bc}	1.15^a	1.49^a
Grassland	0.23^a	0.25^a	0.49^a	0.98^{ab}	0.35^a	0.41^a
Pine	0.24^a	0.24^a	0.40^a	0.72^a	0.40^a	0.44^a
P values (Land use/Temperature)	0.796		**0.048**		0.809	

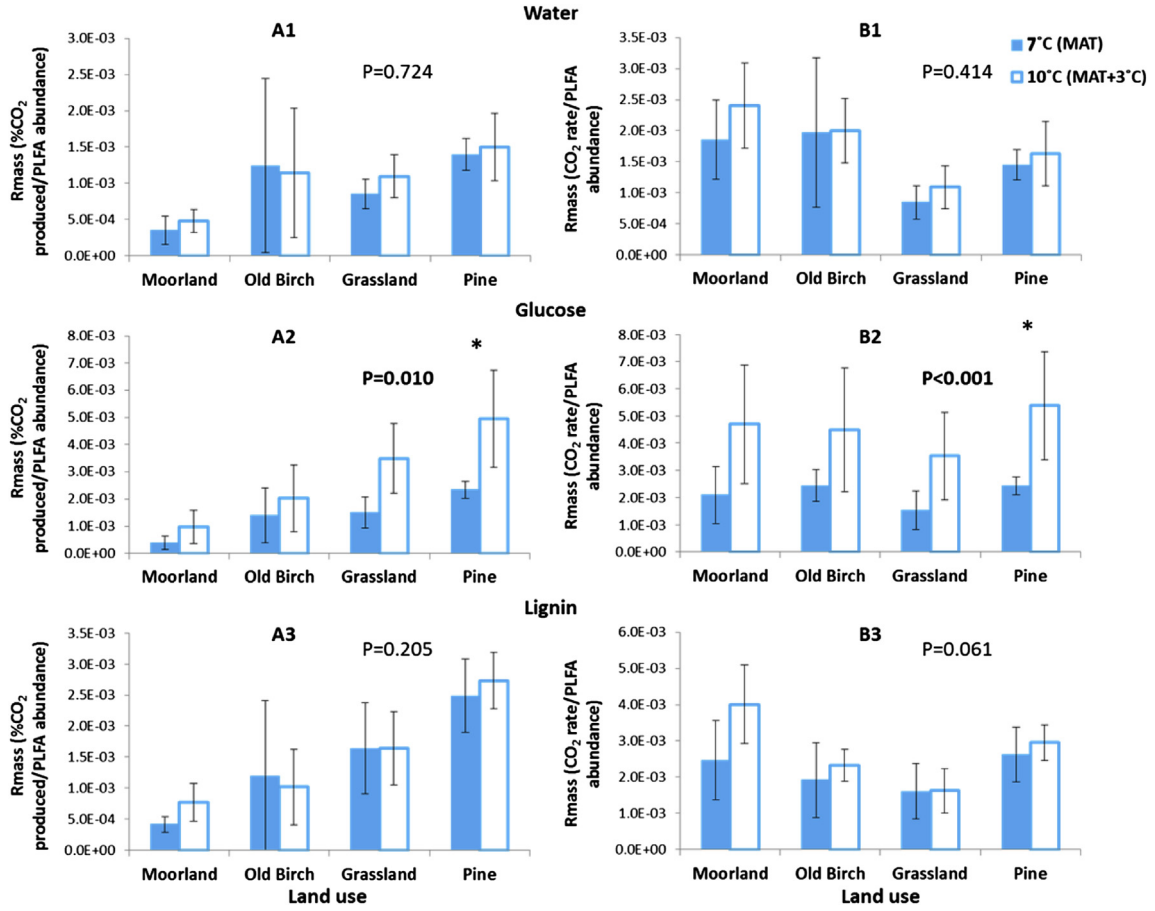

Fig. 4. Effect of land use and temperature on mass-specific soil respiration (Rmass) after 24 h of incubation. Rmass was calculated by dividing the respiration rates (see Supplementary Fig. 2) by the total PLFA abundance (see Supplementary Fig. 3). For each carbon source/substrate (*i.e.* water (1), glucose (2) and lignin (3)), data represent the predicted means (n = 8 for Moorland and Old Birch; n = 4 for Grassland and Pine) and P values obtained from LMM analysis. Graphs labelled (A) are based on the cumulated percentage of CO_2 produced, while graphs labelled (B) are based on the calculated CO_2 rates. Error bars represent standard deviations. The asterisks show the treatments for which a significant difference was detected by the post-hoc test (Bonferroni procedure – $\alpha < 0.05$).

samples. Again, the strongest and statistically significant increase in Rmass was observed for soils amended with glucose (Fig. 4).

Using PLFA-SIP, we investigated the microbial activity when a labile (^{13}C-glucose) or recalcitrant (^{13}C-lignin) substrate was added (water was also used as control) and when incubated at MAT and MAT + 3 °C. Analysis of the PLFA data using PCA-CVA showed that land-use change (afforestation) altered microbial communities although it was statistically significant only at the moorland sites in both absence and presence of C substrate amendment ($P < 0.001$; Fig. 5 and Supplementary Table 6). However, the increase in temperature (MAT + 3 °C) did not change the microbial composition in any of the land uses (Fig. 5 and Supplementary Table 6).

Further analysis of the PLFA data showed that the shifts in community composition due to land-use change were congruent with changes in the abundance of some of the PLFAs (Supplementary Fig. 3). Birch afforestation of moorland decreased the amount of total PLFA ($P < 0.001$), in particular the PLFAs characteristic to Fungi, Gram positive and Gram negative bacteria ($P < 0.001$, Supplementary Table 7). Moreover, the Fungi:Bacteria PLFA ratio was highly informative as it indicated that upon moorland afforestation by birch a loss of Fungi PLFA occurred (Supplementary Fig. 3). No significant effect of temperature was noted on the abundance or pattern of PLFA profile. This was further confirmed by the analysis of active microbial communities (^{13}C-enriched PLFA) obtained from two incubation temperatures. Together with the lack of temperature effect on the amount of ^{13}C

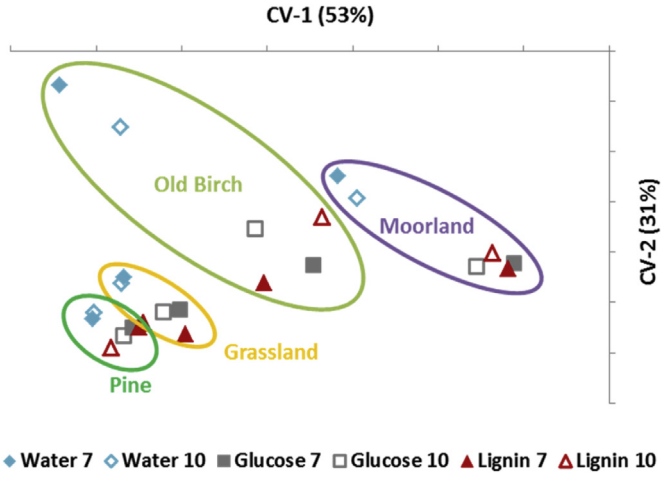

Fig. 5. Microbial composition in soils after incubation without substrate and with the substrates ^{13}C-glucose and ^{13}C-lignin under MAT (7 °C) and MAT + 3 °C (10 °C). Data represent the means of the canonical variates (CV) scores of each soil sample (n = 8 for Moorland and Old Birch; n = 4 for Grassland and Pine) based on the analysis of the PC scores (data not shown) obtained from the % of total PLFA on a mole basis. Only PLFAs that constituted >1% of the total PLFA abundance were included in the principal component analysis (PCA), representing >94% of PLFA presence in all samples. The number of dimensions retained (first six PC axes) for the canonical variates analysis (CVA) was determined by analysis of a scree plot and represented ~90% of the total variation. The circles do not represent any statistical difference between treatments. They were added for clarity only. The significance tests are presented in Supplementary Table 6.

enrichment in total and individual PLFAs, our data showed that the warming treatment did not alter the structure of the active microbial community nor did it result in a higher C incorporation in microbial biomass (carbon-use efficiency) even in the presence of an excessive supply of C substrates. This observation was further confirmed by comparing microbial community structure using MT-RFLP and 454 pyrosequencing. Analysis of MT-RFLP data showed that fungal, bacterial and archaeal communities were strongly influenced by land-use types (Fig. 6; $P < 0.001$). Supply of excessive glucose and lignin also impacted the fungal and, to a lesser extent, archaeal communities but temperature warming did not have any impact on any of the microbial community structures (Supplementary Table 8). Data from 454 pyrosequencing confirmed the results obtained from both PLFA-SIP and MT-RFLP, where the relative abundance of any bacterial phylum did not shift significantly due to temperature warming treatments (Supplementary Figs. 4 and 5).

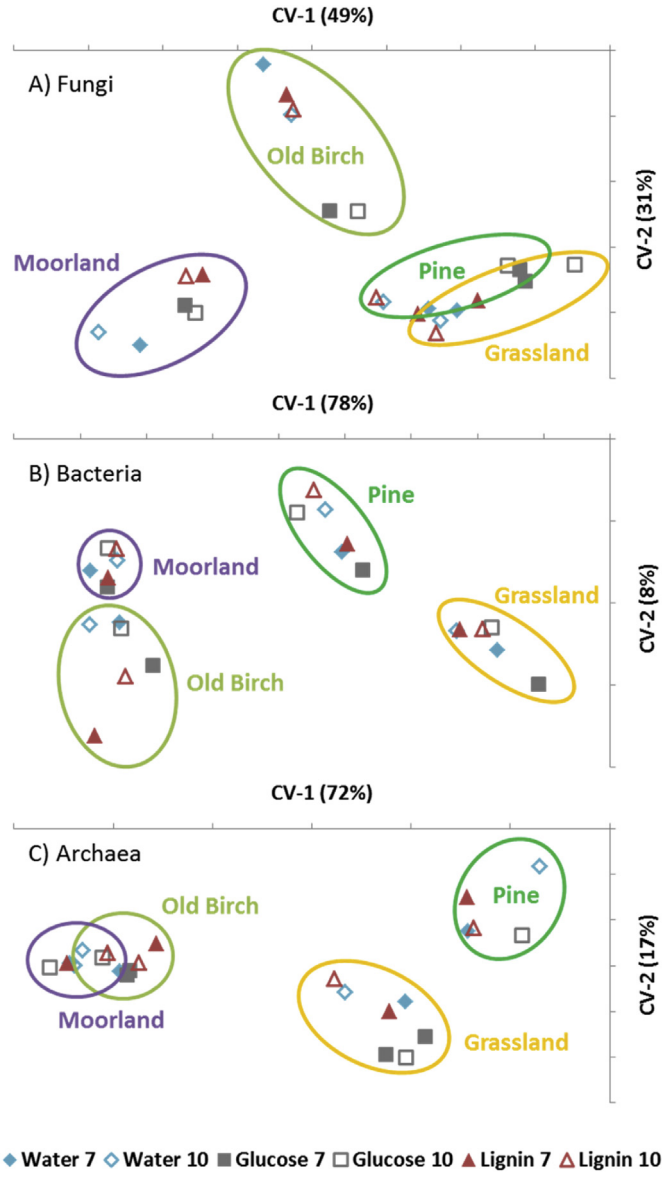

Fig. 6. The effect of land use on the microbial community structure after incubation without substrate and with ^{13}C-glucose and ^{13}C-lignin under MAT (7 °C) and MAT + 3 °C (10 °C). For each microbial group (Fungi (A), Bacteria (B) and Archaea (C)), data represent the means of the canonical variates (CV) scores of each soil sample ($n = 8$ for Moorland and Old Birch; $n = 4$ for Grassland and Pine) based on the analysis of the PC scores (data not shown) obtained from the MT-RFLP. The number of dimensions retained (first eight PC axes for Fungi and Bacteria, and first five PC axes for Archaea) for the canonical variates analysis (CVA) was determined by analysis of a scree plot and represented ~80% (Fungi), 90% (Bacteria) and 95% (Archaea) of the total variation. The circles do not represent any statistical difference between treatments. They were added for clarity only. The significance tests are presented in Supplementary Table 8.

4. Discussion

4.1. Land use impact on soil respiration and microbial community structure

It is well established that the response of Rs due to land-use change is linked to shifts in soil abiotic and biotic properties (Singh et al., 2010). In this study, we first investigated two types of land-use changes: the afforestation of moorland by birch trees and the afforestation of grassland by pine trees. Our data provided a contradictory effect of afforestation on Rs. While afforestation of moorland increased Rs, afforestation of grassland significantly reduced Rs. A number of previous studies reported reductions in Rs upon afforestation of pasture and cropland (Scott et al., 2006; Scott-Denton et al., 2006; Hiltbrunner et al., 2012, 2013; Wang et al., 2013; Zhang et al., 2013) that was attributed to low C inputs from tree to belowground communities. This observation is consistent with a previous report of considerably lower primary production and soil respiration in forest compared with grassland at the field scale (Schulze et al., 2010). However, we found the total opposite response of Rs to moorland afforestation, which also resulted in a substantial loss of SOC under birch woodlands. The fact that increased Rs under a birch forest linked to a significant loss in SOC suggests that the introduction of birch trees led to increased microbial activity which in turn increased Rs. Loss of SOC under afforestation was reported previously with a link to low C inputs in soils from trees (Risch et al., 2008). Our data suggest that the characteristics of trees (*i.e.* birch *vs.* pine) can also impact SOC and Rs through their influence on microbial communities and their activities. Our observation was supported by a previous study by Nielsen et al. (2010) which reported a consistent negative impact of birch afforestation/colonisation on SOC at 12 sites across Scotland.

Analysis of the soil characteristics investigated in this study showed that sites with contrasting land use had vastly different abiotic (*e.g.* pH, total C and N, C:N ratio, moisture) and biotic (microbial community composition) properties. A number of previous studies have reported that land-use change led to significant shift in microbial community composition which could be linked to changes in soil abiotic properties such as pH, total C and C quality (Grayston et al., 1998; Kuske et al., 2002; Costa et al., 2006; Macdonald et al., 2009). For example, using PLFA and MT-RFLP analyses, Macdonald et al. (2009) reported that the afforestation of pasture with pine trees strongly impacted microbial communities. Nazaries et al. (2013), using the same sites as in this study, reported a significant impact of afforestation on methane oxidation and methane-oxidising microbial communities. Surprisingly, we found contrasting observation here too. While the fungal community was more responsive to the afforestation of moorland by birch woodland, the bacterial and archaeal communities responded strongly to the afforestation of grassland by pine tree. This, again, highlights the fact that characteristics of tree species can influence microbial community compositions which is consistent with previous findings on the impact of moorland afforestation on soil microbial communities (Nielsen et al., 2010). One key finding in our study is that both fungal biomass and community structure were negatively impacted by birch afforestation which may explain the higher Rs and significant loss of SOC observed under birch

woodlands. Previous reports documented the positive relationship between fungal biomass and SOC (Six et al., 2006).

Our 454 pyrosequencing data supported the MT-RFLP results. The three sites investigated had very different types of microorganisms present with the phylum Acidobacteria dominating the Craggan and Tulchan sites (Moorland/Old Birch) whereas the Glensaugh site (Grassland/Pine) was characterised by a higher dominance of the phyla Firmicutes and Proteobacteria (in particular the classes Alphaproteobacteria and Gammaproteobacteria). However, all four land-use types contained a high proportion of Actinobacteria. Afforestation of grassland and moorland increased the relative abundance of Acidobacteria and Verrumicrobiota, respectively. Additionally, afforestation of moorland resulted in a decline in the relative abundance of Actinobacteria while Proteobacteria abundance declined in the pine forest. Our results are consistent with previous reports which found shifts in microbial composition due to afforestation (Macdonald et al., 2009; Nazaries et al., 2013).

To identify which abiotic and biotic factors explained the changes in Rs due to land use, we used information theory and multi-model inference. It revealed that the changes in Rs observed across our four land uses could be best explained by changes in soil properties such as pH, C:N ratio, moisture and inorganic N content (ammonium and nitrate), which is consistent with previous findings (Scott et al., 2006; Macdonald et al., 2009). Another key driver of Rs was the microbial community structure, in particular the bacterial and, to some extent, the archaeal composition. Removing one or more of the soil properties or the bacterial community from the model greatly reduced its performance. This latter finding demonstrates the importance to include microbial data in models that aim to predict ecosystem functioning and biogeochemical cycles, which usually rely mainly on climo-edaphic properties. Within this context, our findings are consistent with those of Powell et al. (2015), who showed the role played by microbial diversity in enhancing the quality of predictive models linking environmental parameters to ecosystem properties.

4.2. Response of soil respiration to climate warming as modulated by land-use type and availability and type of substrates

We tested three proposed mechanisms for the temperature sensitivity of Rs (Singh et al., 2010): (1) loss of labile C (substrate depletion theory); (2) change in microbial physiology; or (3) change in microbial community composition. Previous studies suggested that Rs acclimation can be either explained by a single factor of substrate depletion (Kirschbaum, 2004; Knorr et al., 2005) or by both substrate depletion and microbial acclimation (Allison et al., 2010). We found a consistent increase in Rs and Rmass under elevated temperature, which was further enhanced in the presence of labile C (glucose), irrespective of land use types or the locations from where samples were collected. However, the magnitude of Rs was higher in soils rich in SOC (moorland and birch). Our findings are consistent with a number of recent studies on this topic, irrespective of the duration of incubation, *i.e.* short-term *vs.* long-term incubation (Bradford et al., 2008, 2010; Thiessen et al., 2013; Tucker et al., 2013; Karhu et al., 2014). The rate of respiration subsided after 24 h in all samples except for soils supplied with glucose. This indicates that the response of Rs to warming treatment was modulated by the availability of labile carbon and provides support for the substrate depletion theory (Hartley et al., 2007). We used this short-term incubation study to identify Rs response to warming treatment because it is similar to approaches used extensively in plant, animal and microbe acclimation studies (Atkin and Tjoelker, 2003; Bradford et al., 2008; Tjoelker et al., 2008; Tucker et al., 2013). Additionally, our approach allowed us to investigate the response of Rs rate in absence (basal Rs) and presence of an excessive concentration of labile (glucose) and recalcitrant (lignin) substrates. The response of Rs from all soils was similar (enhanced) although the magnitude was expectedly different with the rate of Rs in the following order: glucose > lignin > no substrate amended samples. This consistency in the response was surprising given that the native substrate quality (organic matter originated from different land uses), soil types and microbial composition were vastly different in our soils. This finding contradicts previous reports where the temperature sensitivity of Rs can be influenced by tree plantation on a cropland (Lu et al., 2012) but support a recent study of Karhu et al. (2014) which reported an enhanced Rs response in carbon-rich ecosystems.

In our study, microbial biomass, community structure (measured by PLFA and MT-RFLP), diversity and community composition (determined by 454 pyroseqencing) and active microbial population (obtained from PLFA-SIP) did not respond to the warming treatment. Several studies previously reported that warming reduced microbial biomass (Bradford et al., 2008; Weedon et al., 2013). This decline in microbial biomass was linked to either depletion in labile C (Knorr et al., 2005; Bradford et al., 2008) or decrease in carbon-use efficiency (Allison et al., 2010; Tucker et al., 2013). However, other studies (Karhu et al., 2014; Wei et al., 2014) did not find any significant decline in microbial biomass nor did its linkage to response of Rs to warming treatments; and our findings are consistent with these reports. Furthermore, we tested if the response of Rs to warming treatment could be explained by microbial community structure. Rs response to climate warming can be explained by two mechanisms linked to microbial community: (1) warming alters the activity of native microbial communities without altering the microbial community structure; (2) warming alters microbial community composition. In both scenarios, Rs response will be altered but in the first scenario, the behaviour and control of Rs rate remains unchanged and thus could allow parameterisation of microbial data in simulation models with comparative ease (Schimel and Gulledge, 1998; Singh et al., 2010). We found no effect of warming treatment on total or active microbial community structure measured by three independent approaches: PLFA-SIP, MT-RFLP and 454 pyrosequencing. This suggests that Rs response to climate warming in our samples can be explained by the first scenario, *i.e.* enhanced microbial metabolic activities without altering the microbial composition (Singh et al., 2010), which supports previous modelling findings (Allison et al., 2010). This finding provides a strong framework for future works which should explicitly include soils types and the cross amendment of the litter to different soils.

5. Conclusion

Overall, we provide evidence of microbial mechanisms for the Rs response to land use and climate warming. Changes in soil respiration following afforestation were dependant on the original soil properties but was also strongly linked to the microbial community structure (mainly bacteria) found in the different types of land use. Increased Rs and Rmass under warming treatment, together with strong positive feedbacks in the presence of glucose, suggest that the Rs response to warming treatment was modulated by the substrate availability and quality (labile *vs.* recalcitrant). A stronger positive response of Rs in SOC-rich samples (moorland/birch woodland sites) suggests that soils with high C content could lose C at higher rates under global warming. No change in total or active (^{13}C-enriched) biomass coupled with no change in microbial community structure under warming treatment in the presence or absence of labile and recalcitrant substrate suggest that, in the

short-term, increased metabolic activity is an important mechanism that can explain the response of Rs to climate warming. Overall, a combination of substrate depletion and increased metabolic rate mechanisms explained Rs response to warming in our study, and should be taken into account when predicting feedback Rs under predicted global warming.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.06.027.

References

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.
Atkin, O.K., Tjoelker, M.G., 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science 8, 343–351.
Barger, N.N., Archer, S.R., Campbell, J.L., Huang, C.-Y., Morton, J.A., Knapp, A.K., 2011. Woody plant proliferation in North American drylands: a synthesis of impacts on ecosystem carbon balance. Journal of Geophysical Research: Biogeosciences 116, G00K07.
Boschker, H.T.S., Nold, S.C., Wellsbury, P., Bos, D., de Graaf, W., Pel, R., Parkes, R.J., Cappenberg, T.E., 1998. Direct linking of microbial populations to specific biogeochemical processes by ^{13}C-labelling of biomarkers. Nature 392, 801–805.
Bradford, M.A., 2013. Thermal adaptation of decomposer communities in warming soils. Frontiers in Microbiology 4.
Bradford, M.A., Watts, B.W., Davies, C.A., 2010. Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. Global Change Biology 16, 1576–1588.
Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D., 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11, 1316–1327.
Bragg, L., Stone, G., Imelfort, M., Hugenholtz, P., Tyson, G.W., 2012. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nature Methods 9, 425–426.
Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: a Practical Information-theoretic Approach. Springer.
Burnham, K.P., Anderson, D.R., 2004. Multimodel inference understanding AIC and BIC in model selection. Sociological Methods & Research 33, 261–304.
Burnham, K., Anderson, D., Huyvaert, K., 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65, 23–35.
Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.
Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., Smalla, K., 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiology Ecology 56, 236–249.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
De Klein, C.A.M., Harvey, M.J., 2012. Nitrous Oxide Chamber Methodology Guidelines. In: Global Research Alliance on Agricultural Greenhouse Gases. Ministry of Primary Industries, New Zealand. http://www.globalresearchalliance.org/research/livestock/activities/nitrous-oxide-chamber-methodology-guidelines/.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Friedlingstein, P., Cox, P., Betts, R., Bopp, L., Von Bloh, W., Brovkin, V., Cadule, P., Doney, S., Eby, M., Fung, I., 2006. Climate-carbon cycle feedback analysis: results from the C4MIP model intercomparison. Journal of Climate 19, 3337–3353.
Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30, 369–378.
Hartley, I.P., Heinemeyer, A., Ineson, P., 2007. Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. Global Change Biology 13, 1761–1770.
Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M., Wookey, P.A., 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. Ecology Letters 11, 1092–1100.
Hiltbrunner, D., Zimmermann, S., Hagedorn, F., 2013. Afforestation with Norway spruce on a subalpine pasture alters carbon dynamics but only moderately affects soil carbon storage. Biogeochemistry 115, 251–266.
Hiltbrunner, D., Zimmermann, S., Karbin, S., Hagedorn, F., Niklaus, P.A., 2012. Increasing soil methane sink along a 120-year afforestation chronosequence is driven by soil moisture. Global Change Biology 18, 3664–3671.
Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K., Subke, J.A., Wookey, P.A., Agren, G.I., Sebastia, M.T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A.T., Salinas, N., Hartley, I.P., 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. Nature 513, 81–84.
Kirschbaum, M.U., 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10, 1870–1877.
Kirschbaum, M.U., 2006. Temporary carbon sequestration cannot prevent climate change. Mitigation and Adaptation Strategies for Global Change 11, 1151–1164.
Knorr, W., Prentice, I.C., House, J.I., Holland, E.A., 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433, 298–301.
Kuske, C.R., Ticknor, L.O., Miller, M.E., Dunbar, J.M., Davis, J.A., Barns, S.M., Belnap, J., 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Applied and Environmental Microbiology 68, 1854–1863.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71, 8228–8235.
Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective distance metric for microbial community comparison. ISME Journal 5, 169.
Lu, S., Zhang, J., Meng, P., Liu, W., 2012. Soil respiration and its temperature sensitivity for walnut intercropping, walnut orchard and cropland systems in North China. Journal of Food, Agriculture & Environment 10, 1204–1208.
Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh, B.K., 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiata*. Soil Biology and Biochemistry 41, 1642–1651.
Mapanda, F., Mupini, J., Wuta, M., Nyamangara, J., Rees, R.M., 2010. A cross-ecosystem assessment of the effects of land cover and land use on soil emission of selected greenhouse gases and related soil properties in Zimbabwe. European Journal of Soil Science 61, 721–733.
Matthias, A.D., Blackmer, A.M., Bremner, J.M., 1980. A simple chamber technique for field measurement of emissions of nitrous oxide from soils. Journal of Environmental Quality 9, 251–256.
Martins, C.S.C., Nazaries, L., Macdonald, C.A., Anderson, I.C., Singh, B.K., 2015. Water availability and abundance of microbial groups are key determinants of greenhouse gas fluxes in a dryland forest ecosystem. Soil Biology and Biochemistry 86, 5–16.
Mundry, R., 2011. Issues in information theory-based statistical inference: a commentary from a frequentist's perspective. Behavioral Ecology and Sociobiology 65, 57–68.
Nazaries, L., Pan, Y., Bodrossy, L., Baggs, E.M., Millard, P., Murrell, J.C., Singh, B.K., 2013. Evidence of microbial regulation of biogeochemical cycles from a study on methane flux and land-use change. Applied and Environmental Microbiology 79, 4031–4040.
Nielsen, U.N., Osler, G.H.R., Campbell, C.D., Burslem, D.F.R.P., van der Wal, R., 2010. The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. Journal of Biogeography 37, 1317–1328.
Powell, J.R., Welsh, A., Hallin, S., 2015. Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. Ecology 96, 1985–1993.
Rangel, T.F., Diniz-Filho, J.A., Bini, L.M., 2010. SAM: a comprehensive application for spatial analysis in macroecology. Ecography 33, 46–50.
Risch, A.C., Jurgensen, M.F., Page-Dumroese, D.S., Wildi, O., Schütz, M., 2008. Long-term development of above-and below-ground carbon stocks following land-use change in subalpine ecosystems of the Swiss National Park. Canadian Journal of Forest Research 38, 1590–1602.
Ross, D.J., Tate, K.R., Scott, N.A., Wilde, R.H., Rodda, N.J., Townsend, J.A., 2002. Afforestation of pastures with *Pinus radiata* influences soil carbon and nitrogen pools and mineralisation and microbial properties. Soil Research 40, 1303–1318.
Schimel, J.P., Gulledge, J.A.Y., 1998. Microbial community structure and global trace gases. Global Change Biology 4, 745–758.
Schulze, E.D., Ciais, P., Luyssaert, S., Schrumpf, M., Janssens, I.A., Thiruchittampalam, B., Theloke, J., Saurat, M., Bringezu, S., Lelieveld, J., 2010. The European carbon balance. Part 4: integration of carbon and other trace-gas fluxes. Global Change Biology 16, 1451–1469.

Scott, N.A., Tate, K.R., Ross, D.J., Parshotam, A., 2006. Processes influencing soil carbon storage following afforestation of pasture with *Pinus radiata* at different stocking densities in New Zealand. Soil Research 44, 85–96.

Scott-Denton, L.E., Rosenstiel, T.N., Monson, R.K., 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology 12, 205–216.

Singh, B.K., Bardgett, R.D., Smith, P., Reay, D.S., 2010. Microorganisms and climate change: terrestrial feedbacks and mitigation options. Nature Reviews Microbiology 8, 779–790.

Singh, B.K., Nazaries, L., Munro, S., Anderson, I.C., Campbell, C.D., 2006. Use of multiplex terminal-restriction fragment length polymorphism for rapid and simultaneous analysis of different components of the soil microbial community. Applied and Environmental Microbiology 72, 7278–7285.

Singh, B.K., Tate, K.R., Ross, D.J., Singh, J., Dando, J., Thomas, N., Millard, P., Murrell, J.C., 2009. Soil methane oxidation and methanotroph responses to afforestation of pastures with *Pinus radiata* stands. Soil Biology and Biochemistry 41, 2196–2205.

Singh, B.K., Quince, C., Macdonald, C.A., Khachane, A., Thomas, N., Al-Soud, W.A., Sørensen, S.J., He, Z., White, D., Sinclair, A., 2014. Loss of microbial diversity in soils is coincident with reductions in some specialized functions. Environmental Microbiology 16, 2408–2420.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, B., Midgley, B.M., 2013. IPCC, 2013: Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Symonds, M.R., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65, 13–21.

Thiessen, S., Gleixner, G., Wutzler, T., Reichstein, M., 2013. Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass: an incubation study. Soil Biology and Biochemistry 57, 739–748.

Thornton, B., Zhang, Z.L., Mayes, R.W., Hogberg, M.N., Midwood, A.J., 2011. Can gas chromatography combustion isotope ratio mass spectrometry be used to quantify organic compound abundance? Rapid Communications in Mass Spectrometry 25, 2433–2438.

Tjoelker, M.G., Oleksyn, J., Reich, P.B., Zytkowiak, R., 2008. Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in *Pinus banksiana* across wide-ranging sites and populations. Global Change Biology 14, 782–797.

Tucker, C.L., Bell, J., Pendall, E., Ogle, K., 2013. Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? Global Change Biology 19, 252–263.

Vicca, S., Fivez, L., Kockelbergh, F., Van Pelt, D., Segers, J.J., Meire, P., Ceulemans, R., Janssens, I.A., 2009. No signs of thermal acclimation of heterotrophic respiration from peat soils exposed to different water levels. Soil Biology and Biochemistry 41, 2014–2016.

Wagner, S.W., Reicosky, D.C., Alessi, R.S., 1997. Regression models for calculating gas fluxes measured with a closed chamber. Agronomy Journal 89, 279–284.

Wang, W., Zeng, W., Chen, W., Zeng, H., Fang, J., 2013. Soil respiration and organic carbon dynamics with grassland conversions to woodlands in temperate China. PLoS One 8, e71986.

Waldrop, M.P., Harden, J.W., 2008. Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest. Global Change Biology 14, 2591–2602.

Weedon, J.T., Aerts, R., Kowalchuk, G.A., van Logtestijn, R., Andringa, D., van Bodegom, P.M., 2013. Temperature sensitivity of peatland C and N cycling: does substrate supply play a role? Soil Biology and Biochemistry 61, 109–120.

Wei, H., Guenet, B., Vicca, S., Nunan, N., AbdElgawad, H., Pouteau, V., Shen, W., Janssens, I.A., 2014. Thermal acclimation of organic matter decomposition in an artificial forest soil is related to shifts in microbial community structure. Soil Biology and Biochemistry 71, 1–12.

Xu, Y., Moser, C., Al-Soud, W.A., Sørensen, S.J., Høiby, N., Nielsen, P.H., Thomsen, T.R., 2012. Culture-dependent and -independent investigations of microbial diversity on urinary catheters. Journal of Clinical Microbiology 50, 3901–3908.

Zhang, W., Wang, X., Wang, S., 2013. Addition of external organic carbon and native soil organic carbon decomposition: a meta-analysis. Plos One 8, e54779.