



## Diminished soil functions occur under simulated climate change in a sup-alpine pasture, but heterotrophic temperature sensitivity indicates microbial resilience

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### HIGHLIGHTS

- Climate change threatens sustainable use of mountain pastures by disturbing nutrient and carbon cycling.
- We simulated climate change through transplantation of intact pasture turfs along an elevation gradient.
- Moisture removed in situ temperature sensitivity of respiration.
- Respiration showed variable recovery after wetting, but comparable  $Q_{10}$  values among sites.
- Changes to soil function were seen after crossing a model-realistic climate threshold.

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### ABSTRACT

The pressure of climate change is disproportionately high in mountainous regions, and small changes may push ecosystem processes beyond sensitivity thresholds, creating new dynamics of carbon and nutrient cycling. Given that the rate of organic matter decomposition is strongly dependent upon temperature and soil moisture, the sensitivity of soil respiration to both metrics is highly relevant when considering soil–atmosphere feedbacks under a changing climate. To assess the effects of changing climate in a mountain pasture system, we transplanted turfs along an elevation gradient, monitored in situ soil respiration, incubated collected top-soils to determine legacy effects on temperature sensitivity, and analysed soil organic matter (SOM) to detect changes in quality and quantity of SOM fractions. In situ transplantation down-slope reduced soil moisture and increased soil temperature, with concurrent reductions in soil respiration. Soil moisture acted as an overriding constraint to soil respiration, and significantly reduced the sensitivity to temperature. Under controlled laboratory conditions, removal of the moisture constraint to heterotrophic respiration led to a significant respiration-temperature response. However, despite lower respiration rates down-slope, the response function was comparable among sites, and therefore unaffected by antecedent conditions. We found shifts in the SOM quality, especially of the light fraction, indicating changes to the dynamics of decomposition of recently deposited material. Our findings highlighted the resilience of the microbial community to severe climatic perturbations, but also that soil moisture stress during the growing season can significantly reduce soil function in addition to direct effects on plant productivity. This demonstrated the sensitivity of subalpine pastures under climate change, and possible implications for sustainable use given reductions in organic matter turnover and consequent feedbacks to nutrient cycling.

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### 1. Introduction

Through altering temperature and precipitation regimes, climate change is projected to place increasing pressure on the sustainable use and productivity of semi-natural grassland systems. This is thought to be especially so for mountain grasslands such as those in the alpine

and sub-alpine zones (Dunne et al., 2003). Elevated growing-season temperatures and more frequent occurrence of drought periods have the capacity to alter carbon (C) and nutrient cycling at the ecosystem level (Walther et al., 2002), with potential for significant soil–plant feedbacks (Melillo et al., 2002). Higher temperatures may lead to enhanced C losses from soils (Bond-Lamberty and Thomson, 2010; Kirschbaum, 1995) and faster nutrient cycling (Shaw and Harte, 2001; Ineson et al., 1998; Zhang et al., 2012), yet these increases may be offset, or even reversed by the effect of reduced moisture status (Davidson and Janssens, 2006). Whilst dependent upon antecedent conditions and biological community adaptation, moisture stress, especially during the growing season, can lead to considerable reductions in C fixation, and rates of C and nutrient cycling. This is of particular concern given that soil respiration is the second largest annual flux (after photosynthesis) of C between the atmosphere and terrestrial systems (Houghton, 2007). It is therefore highly relevant to assess change in soil respiration under a changing climate. Soil respiration represents a useful and logistically simple measure of general soil function, as it encapsulates the end product of a range of metabolic processes involved in C and nutrient cycling, as well as organism activity. This is particularly important given that the long-term sustainability of ecosystems depends upon maintaining soil function; therefore respiration provides a broad yet sensitive indicator of change.

The fundamental metabolic dependence upon temperature suggests that rate should increase with temperature according to a simple response function (Arrhenius, 1889). In the case of soil respiration, this must assume that all other constraining factors are not limiting, but in situ, this is rarely the case over any significant spatial or temporal scale. Therefore, when measured in the field, the observed response to temperature is in fact the ‘apparent’ sensitivity, and recognises the myriad drivers that exist. The sensitivity to temperature is often encapsulated by the  $Q_{10}$  function, which essentially describes the change in rate over a 10 °C change in temperature. This function carries the benefit of being simple and comparable among systems, but is influenced by the statistical model used to derive the temperature–respiration fit (Lloyd and Taylor, 1994). Soil moisture also strongly constrains ecosystem function, and its effect on soil respiration, as well as the interaction with temperature, has been the subject of research for a considerable time (Orchard and Cook, 1983; Flanagan and Johnson, 2005; Yuste et al., 2007; Davidson et al., 2000; Xu et al., 2004; Sjögersten et al., 2012). The conceptual moisture–respiration model of Suseela et al. (2012) serves to summarise the occurrence of a moisture optimum, which approximately coincides with field capacity. The broad applicability of such a model, especially along gradients of drying from optimum, is evident (e.g. Chen et al., 2008), and such a relationship provides a basis for modelling dependencies. However, the interaction of soil moisture with temperature requires consideration of multi-factor approaches, and this dual-driving of function by moisture and temperature has been the focus of a substantial research effort (e.g. Davidson et al., 2000; Raich and Tufekciogul, 2000; Janssens and Pilegaard, 2003). This interaction becomes especially apparent when considering how moving away from soil moisture optima can strongly reduce the temperature sensitivity of soil respiration (Suseela et al., 2012; Lellei-Kovács et al., 2011; Sowerby et al., 2008). To assess the intrinsic temperature sensitivity of soil respiration, incubations of soil cores across temperature manipulations (Yuste et al., 2007; Fang and Moncrieff, 2001) can be used. This approach could also provide information on whether apparent sensitivity in situ is the product of real changes to intrinsic sensitivity, or more a function of other constraining factors.

Investigating change in soil function, and its sensitivity to moisture and temperature can be contextualised by considering changes to soil organic matter (SOM) quality and quantity, primarily as an indicator of any trajectories in C accumulation or loss. Whilst SOM is an inherently complex material that exists along a decomposition continuum, fractions of SOM that reflect early stages of decomposition can be separated

to serve as a more sensitive indicator to short-term change than bulk SOM. This is usually carried out by density separation, where a light fraction organic matter is separated (Sollins et al., 1984; Leifeld and Kögel-Knabner, 2005; Schindlbacher et al., 2010), and is assumed to be relatively available due to a lack of mineral interactions or aggregate occlusions. Not only does this fraction serve as a sensitive indicator of change, but the quality of the light fraction could also be related to temperature sensitivity (Schindlbacher et al., 2010). This relationship can be used to explore whether changes in temperature sensitivity of soil respiration are related to so-called ‘thermal acclimation’ (Bradford et al., 2008), or to changes in substrate quality (Fierer et al., 2005), which is central to respiration–temperature debate (Subke and Bahn, 2010).

Much experimental work to consider the role of climate change on soil and plant function has been based on in situ manipulations, yet natural climate gradients can also be used to study spatial variation in ecosystem processes (Emmett et al., 2004; Zhang et al., 2012). Using the established natural gradient of mean annual temperature (MAT) decrease and a precipitation (MAP) increase with higher elevation in mountain regions, one can conveniently substitute space for time in ecological climate change research (Körner, 2007). Soil monolith transplantation from high to low elevations has been shown to effectively simulate a warmer climate in various studies (see Hart and Perry, 1999; Ineson et al., 1998; Link et al., 2003; Olofsson, 2001; Sebastia, 2007), and across much larger spatial scales in general climate change contexts (Breeuwer et al., 2010). Such an approach provides a natural year-round experimental warming, which also accounts for associated changes in precipitation, snow-to-rain ratio, snowmelt, and length of the vegetation growing-season. Transplantation also allows combined temperature and moisture (multi-factor) treatments to be incorporated without possible issues relating to infrastructure effects and uneven treatment effect throughout the year (Carlyle et al., 2011).

To assess the impact of experimental climate change on soil respiration and SOM in sub-alpine pastures, we used an existing mesocosm transplantation experiment in the Swiss Jura Mountains (Gavazov et al., 2013a) where soil and vegetation were transplanted to four elevations representing a climate gradient in moisture and temperature. Soil respiration was measured in situ during the growing season of 2011, and we explored the relationships to moisture and temperature, and the apparent temperature sensitivity. To assess the intrinsic temperature sensitivity of the heterotrophic component of soil respiration, we sampled the uppermost organic layer of the soil and carried out a laboratory incubation across a temperature range. We also considered change to chemical characteristics of the soil organic matter, which we assessed on bulk soil, and on a light fraction of SOM separated by density. The transplantation approach used in this study spans the whole range of scenarios outlined by Meehl et al. (2007) and incorporates expected changes to precipitation for Switzerland (Frei et al., 2006). This set-up allowed for the detection of thresholds in response parameters, i.e. tipping points (see Lenton, 2011), which when reached, can trigger rapid changes in ecosystem function, with feedbacks to ecosystem resilience and sustainable use.

Given the observation of changes in community composition and productivity (Gavazov et al., 2013b), and the established driving of soil respiration by moisture, we first hypothesised that respiration would be reduced at transplantation sites as a function of soil moisture. Secondly, soil temperature was expected to have a control over soil respiration, but the strength of the relationship was hypothesised to reduce down the transplantation gradient in response to increasing moisture stress. For the laboratory incubations, we expected that removal of moisture constraints would establish temperature sensitivities among all soils, and that the strength of this relationship might vary as a function of in situ conditions. Finally, we assumed that lower productivity down-slope would result in lower abundance of light fraction organic matter,

and a lower quality of the separated light fraction (i.e. higher CN ratio).

## 2. Materials and methods

### 2.1. Site description and experimental design

Turfs of mountain pastures were transplanted (in 2009) from the initial donor site in Combe des Amburnex, Switzerland (N 46°55', E 6°24') at 1350 m a.s.l. to four recipient sites: the disturbance control at 1350 m a.s.l. (Marchairuz, N 46°54', E 6°23'), and the downslope sites at 1010 m a.s.l. (St George, N 46°52', E 6°26'), 570 m a.s.l. (Arboretum d'Aubonne, N 46°51', E 6°37') and 395 m a.s.l. (Bois Chamblard, 46°47', E 6°41'). The donor site is a typical pasture of the Jura crest, having a poorly defined, immature O horizon and an undifferentiated, clay-rich B horizon to approximately 20 cm, below which lies a C horizon of limited depth (10–20 cm) before parent material (limestone). The poor resolution of the surface horizons is thought to be due to periodic disturbance by moles and earthworms, and cattle trampling. Soils exhibit signs of hydromorphic processes (iron deposits on relic root channels, and blue-grey mottles on deeper sections). Details of the transplantation procedure, which forms part of an on-going CCES-MOUNTLAND research initiative (<http://www.cces.ethz.ch/projects/sulu/MOUNTLAND>), can be found in Gavazov et al. (2013a). Briefly, each site received five replicated pasture turfs measuring 60 × 80 cm and 35 cm in height (made up of 12 blocks to form the single mesocosm), which were contained within lysimeter mesocosms of the same dimensions. At each site, mesocosms were arranged in a common garden by being installed down level to the ground surface so as to avoid lateral heat exchange. In each mesocosm, ECH<sub>2</sub>O EC-TM sensor probes coupled to Em50 data-loggers (Decagon Devices, Inc., USA) recorded soil temperature and volumetric water content at top-soil horizon (–3 cm) every minute, and data were averaged over one hour intervals. Parameters vary along the transect as a simulation of climate change, spanning a gradient from wet and cold (subalpine zone) to warm and dry (colline zone) growing season conditions.

### 2.2. In-situ soil respiration

Soil respiration was measured approximately fortnightly throughout the snow-free period using a Li-Cor LI8100 infrared gas analyser (IRGA) on a permanent 10 cm PVC collar cut ~2 cm into the soil. Enclosures lasted 2 min with a 30 second dead-band, and flux estimates were calculated automatically after data inspection using a non-linear regression. Fluxes were expressed per unit area and were a mean of the five replicated pasture mesocosms.

### 2.3. Soil sampling and preparation

Soil cores were taken in June 2011 from five replicated mesocosms of the open pasture system at each of the four transplantation sites. The top 4 cm of soil was sampled from the centre of each mesocosm, with a 5 cm diameter steel corer, with vegetation being cut and removed prior to sampling. Soil was stored in a cooled box and transported to the laboratory for temporary storage at 4 °C until analysis. Soils were weighed and measured (for volume calculation) before being lightly homogenised by hand, and having large root (>2 mm) material removed by hand. Soils were not sieved, so as to reduce disturbance, especially to fungal components. For soil chemical analysis, a 5 g subsample was dried at 60 °C and ball milled. For respiration analysis, a ~10 g subsample of soil was adjusted to 100% of water holding capacity (WHC) by addition of deionised water (the quantity being determined previously on separate pasture cores by wetting and draining under gravity to determine WHC per unit of mass) and incubated at 5 °C for 24 h in an unsealed 50 ml polypropylene centrifuge tube prior to respiration analysis.

### 2.4. Respiration analysis

After the 24 h settling period at 5 °C, adjusted soils were subjected to a temperature ramp from 5.5 to 30.5 °C, at 5 degree intervals. Each incubation period lasted for 1.5 h, after which respiration was determined by closing the centrifuge tube and connecting, via tubing, to a Li-COR 8100 infrared gas analyser. Centrifuge tubes were sampled at random on each temperature sampling-occasion, where headspace CO<sub>2</sub> was allowed to accumulate for 60 s and the ppm concentration was automatically recorded every second. After sampling, tubes were returned to the incubator at the next temperature. Respiration rates were calculated using the slope from a non-linear regression to raw ppm data and converting to a mass flux via a simple equation using the ideal gas law, taking into account incubation temperature and the volume of the centrifuge tube plus gas lines, whilst assuming atmospheric pressure of 1 atm. Initial variation in ppm data were excluded to give a 10–15 s dead band using the Li-COR regression analyser tool. Fluxes were expressed as a function of dry soil mass. Temperature dependence of soil respiration was estimated by fitting a simple non-linear model to the derived flux rates (Eq. (1)) and calculation of a Q<sub>10</sub> function (Eq. (2)).

$$R = ae^{(bT)} \quad (1)$$

where R is the flux (ppm CO<sub>2</sub> per second), T is the temperature, and a and b are parameters of the fitted data.

$$Q_{10} = e^{(b10)} \quad (2)$$

### 2.5. Soil physico-chemical analysis

Soil C and N were determined under high-temperature oxidation using a CN analyser (CE Instruments model NA2500 Nitrogen Carbon Analyser). Ca, Mg, Al, K and Fe were determined using atomic absorption spectroscopy (Perkin Elmer AAnalyst 100) after nitric acid digestion. Total phosphorus was determined using flow injection analysis (Foss FIA-star 5000 auto analyser) after digestion in sulphuric acid with hydrogen peroxide. To obtain a light fraction SOM<sub>lf</sub>, 20 g samples of field-moist soil were mixed for 1 h in a 1.2 g cm<sup>-3</sup> solution of sodium iodide (NaI), and then centrifuged at 4000 rpm after which the material floating on the surface was separated by decantation. This procedure was repeated and separated fractions were washed in deionised water, dried and weighed. Total C and N contents of fractions were analysed as for bulk soils after ball milling. Soil moisture and organic matter were determined gravimetrically at 105 °C and 375 °C respectively.

### 2.6. Statistical analyses

The effects of experimental climate change on in situ field respiration rates, as well as on daily values of soil temperature and moisture, were analysed using linear mixed effects (LME) models for repeated measures, with site as a fixed factor with four levels (i.e. elevation of transplantation). Incubation flux estimates were analysed for between-site differences using a LME model with site as a fixed factor, temperature as a continuous co-variable, and their interaction term. Significant differences among individual transplantation sites were determined using linear combinations of contrasts, thus accounting for the model's nested structure. Between and within-site effects of concurrent soil temperature and soil moisture on in situ soil respiration rates were determined using simple linear regression models. Between-site differences in soil physico-chemical characteristics were tested using analysis of variance (ANOVA), followed by Tukey's HSD *post-hoc* tests. Linear associations among edaphic variables were assessed via Pearson's correlation coefficients. Assumptions of normality and homoscedasticity of the residuals



in all final models were verified visually using diagnostic plots, with log and square-root transformations being applied to data when necessary. All analyses were carried out using R v2.13 (R development core team, 2012).

### 3. Results

#### 3.1. Soil micro-climate

Transplantation of monoliths had substantial effects on the soil micro-climate (Fig. 1) during the period May–September (inclusive). Significant overall differences were found among all sites for soil temperature ( $F_{3,16} = 293.7$ ,  $P < 0.001$ ), and for soil moisture ( $F_{3,16} = 22.6$ ,  $P < 0.001$ ), all transplantation sites were significantly different from the control site at 1350 m. All sites experienced substantial fluctuations in both metrics, and variations among sites in soil moisture were reasonably correlated (all  $r > 0.62$ ), but much more so for temperature (all  $r > 0.84$ ).

#### 3.2. Soil chemistry

A summary of selected chemical variables and broad measures of macronutrient and carbon content and ratios are presented in Table 1. Comparison of total C, N and P contents among sites showed comparable values, and therefore ratios of key macronutrients and C remained similar for bulk soil. Soil pH was reduced at all sites relative to control, and significantly lower at sites 395 m and 1010 m than the control site at 1350 m ( $P = 0.016$ ,  $0.002$  respectively). This reduction in pH correlated strongly with changes in Ca ( $r = 0.84$ ) across all sites. Lower levels of Ca at 570 ( $P = 0.019$ ) and 395 ( $P = 0.013$ ) than 1350 m also coincided with higher within-site correlations between Ca and pH (570 m,  $r = 0.97$ , 395 m,  $r = 0.84$ ). Variation among sites in cation content (Table 1) was dominated by significant differences

**Table 1**

Soil physical and chemical characteristics. Values are means, with standard deviation in brackets for the four study sites with elevation shown in metres above sea level.

	1350 m a.s.l	1010 m a.s.l	570 m a.s.l	395 m a.s.l
Bulk density ( $\text{g cm}^{-3}$ )	0.54 (0)	0.6 (0)	0.56 (0.2)	0.59 (0)
pH ( $\text{CaCl}_2$ )	5.4 (0.2)	4.6 (0.4)	5 (0.2)	4.7 (0.4)
C %	10.4 (1.1)	10.1 (3.1)	11.6 (1.3)	9.4 (2)
N %	1 (0)	1 (0.2)	1.1 (0.2)	0.9 (0.2)
C/N	10.5 (0.7)	10.1 (1.8)	10.2 (0.9)	10 (2)
P ( $\text{mg g}^{-1}$ )	1 (0)	1 (0.2)	1.1 (0.2)	0.9 (0.2)
Mg ( $\text{mg g}^{-1}$ )	4.4 (1.3)	4.1 (0.7)	4.6 (0.7)	4.2 (0.4)
Fe ( $\text{mg g}^{-1}$ )	44.5 (2.9)	30.4 (3.6)	34.9 (7.6)	38.7 (4)
K ( $\text{mg g}^{-1}$ )	4.8 (0.4)	3.6 (0.4)	4.2 (0.9)	4 (0.2)
Ca ( $\text{mg g}^{-1}$ )	7.2 (2.2)	3.7 (2)	5.3 (1.6)	3.5 (0.9)
Al ( $\text{mg g}^{-1}$ )	33 (2.7)	24.6 (3.4)	29.6 (4.9)	27.5 (2.2)

between site 1350 m and 1010 m in Al ( $P = 0.011$ ), Fe ( $P = 0.006$ ) and K ( $P = 0.036$ ), with Mg following no apparent trend.

#### 3.3. Soil organic matter light fraction

Light fraction organic matter ( $\text{SOM}_{\text{lf}} < 1.2 \text{ g cm}^{-3}$ ) separated by density fractionation made up a small (<4%) proportion of the total soil mass (Table 2), but in some cases over 10% of the total soil C was found in this fraction. The N content of the  $\text{SOM}_{\text{lf}}$  was typically <7% of total soil-N. The quality of the  $\text{SOM}_{\text{lf}}$  showed it to be only partially decomposed, retaining a relatively high CN of ~18. The variation among sites in the quantity and quality of the  $\text{SOM}_{\text{lf}}$  (Table 2) shows a consistent pattern, with transplanted sites generally containing more  $\text{SOM}_{\text{lf}}$ , with higher C and N contents than that at the control site at 1350 m. Between-site differences though were non-significant for all comparisons apart from the N content between sites 1350 m and 395 m ( $P = 0.02$ ). Despite the lack of significance, there is an appreciable accumulation of  $\text{SOM}_{\text{lf}}$  in transplanted soils, in some cases twice that than at the control.

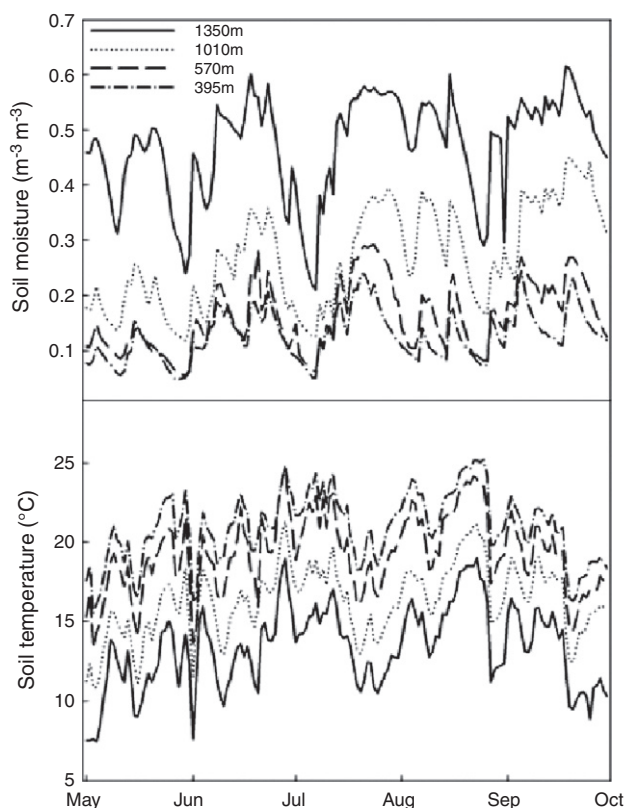
#### 3.4. In situ respiration response to moisture and temperature

Soil transplantation resulted in successive reductions in average soil respiration rates down the elevation gradient for the period May–September 2011. There was a significant effect of site on soil respiration across the period ( $F_{3,16} = 19.6$ ,  $P < 0.001$ ), and as shown in Fig. 2, between site differences were evident among all sites apart from between 570 and 395 m. Overall, lower soil moisture content at transplantation sites coincided with lower respiration rates during the sampling period (Fig. 3), and when all sites were grouped, moisture gave a strong and significant effect ( $R^2 = 0.51$ ,  $P < 0.001$ ). At the site level, moisture had an increasing explanatory power at successive downslope sites (1350  $R^2 = 0.05$ , 1010  $R^2 = 0.14$ , 570  $R^2 = 0.41$ , 395  $R^2 = 0.66$ ), but only at the lowest elevation site (395) was the regression fit significant ( $P = 0.008$ ). Soil temperature (Fig. 4) had no overall significance across sites when data were combined ( $P = 0.28$ ), and at the site level, only the control site at 1350 m gave a significant response ( $R^2 = 0.85$ ,  $P < 0.001$ ), with the transplantation sites all showing non-significant fits with low coefficients of determination ( $R^2 < 0.1$ ).

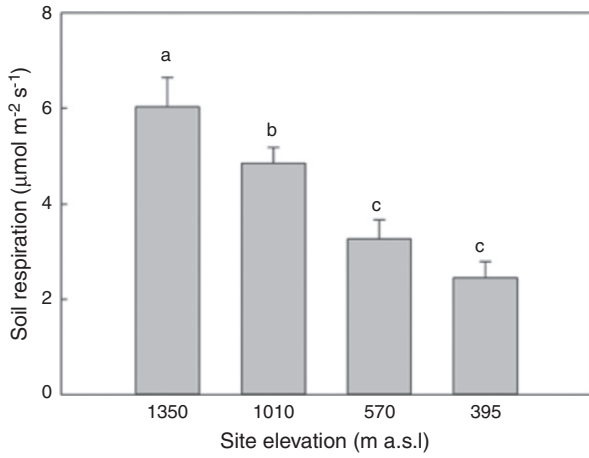
**Table 2**

Mass and chemical variables for the light fraction of soil organic matter obtained by density. Values are means, with standard deviation in brackets for the four study sites with elevation shown in metres above sea level.

	1350 m a.s.l	1010 m a.s.l	570 m a.s.l	395 m a.s.l
Mass % total mass	1.4 (1.6)	2.4 (1.3)	3.2 (1.6)	3 (1.6)
C %	28.7 (4.7)	30.6 (4)	31.6 (1.3)	31.4 (2.5)
N %	1.4 (0)	0.7 (0)	1.8 (0.2)	1.8 (0.2)
LF-C % SOC	3.4 (3.1)	6.1 (1.3)	8.9 (4.2)	9.7 (2.9)
LF-N % soil-N	2 (2.2)	3.3 (0.9)	5.4 (2.9)	5.6 (2.5)
C/N	20.3 (4.2)	18 (2.5)	17.4 (2.2)	17.8 (1.3)



**Fig. 1.** Daily mean soil moisture and temperature across the study period (May–September 2011) for the four study sites with elevation shown in metres above sea level.



**Fig. 2.** Mean soil respiration in situ during the growing season 2011. Letters denote significant differences between sites ( $P < 0.001$ ), for the four study sites with elevation shown in metres above sea level.

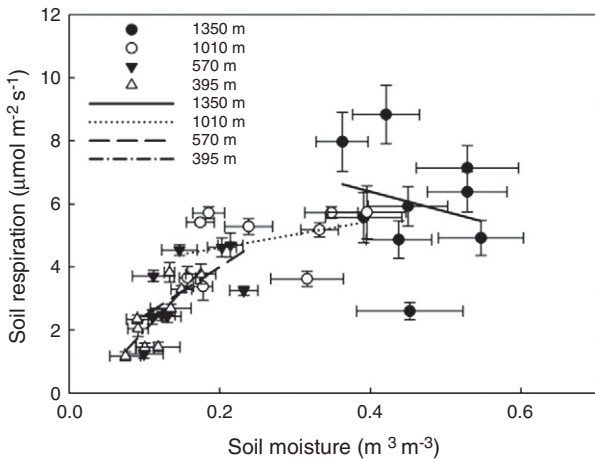
### 3.5. Temperature manipulations

Across the temperature ramp imposed under standardised moisture conditions, Fig. 5 shows sensitivity to temperature among all sites, this being supported by the LME model with an overall temperature effect on flux ( $F_{1,93} = 209.4, P < 0.001$ ). When considering the within site effect, the LME model gave a non-significant interaction term and a low F statistic ( $F_{3,93} = 1.48$ ) for *site:temperature* interaction, therefore unlike the in situ measures, all sites displayed a comparable sensitivity to temperature. Calculation and comparison of  $Q_{10}$  values (Table 3) among sites also gave a non-significant output, reinforcing the similarity of responses when moisture limitation is removed. Despite the similarity in sensitivity response, the magnitude (Fig. 5) of the flux varied among sites ( $F_{3,16} = 9.5, P = 0.008$ ).

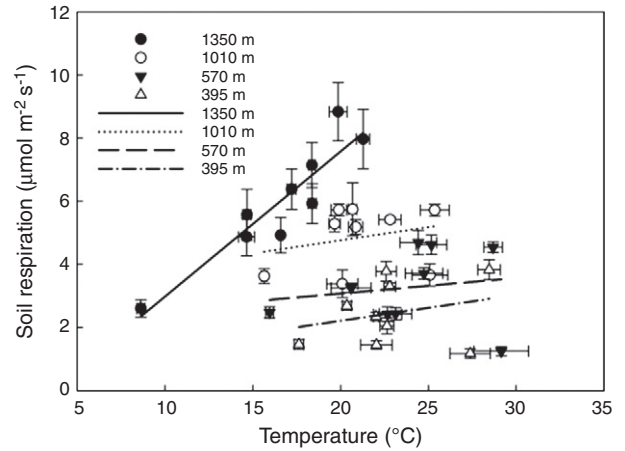
## 4. Discussion

### 4.1. Moisture and temperature effects on in situ soil respiration

To consider the effect of transplantation of pasture soils along a climate gradient on soil function, we measured rates of respiration in situ and used soil moisture and temperature data to explore the relative strength of each driver. Here, we expected a similar change for soil function as was observed for plant productivity by Gavazov et al. (2013b),



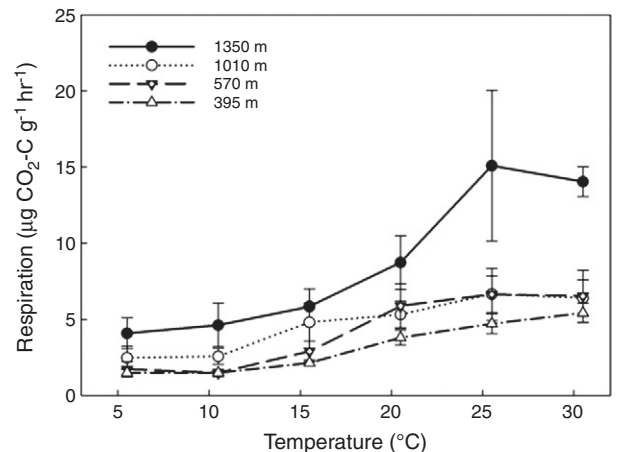
**Fig. 3.** In situ soil respiration and soil moisture during the growing season 2011. Values are means  $\pm$  SEM. Lines indicate regressions, for which coefficients can be found in text, elevation is shown in metres above sea level.



**Fig. 4.** In situ soil respiration and soil temperature during the growing season 2011. Values are means  $\pm$  SEM. Lines indicate regressions, for which coefficients can be found in text, elevation shown in metres above sea level.

i.e. a general reduction at transplantation sites from that of the control site. Specifically, we hypothesised that soil respiration would be constrained along the transplantation gradient in concert with soil moisture. Indeed, this hypothesis was supported as we observed a trend of sequentially lower soil respiration along the gradient, and this reduction coincided with lower soil moisture along the gradient, this being most heavily evident at the driest site, where moisture also significantly explained within-site variation in respiration. When viewed across all sites, our data displayed an expected trend from optimum moisture conditions to extremely water-limited conditions, which fits with the general conceptual moisture-respiration model of Suseela et al. (2012), albeit on the optimum to dry side of the bell-curve.

Given moisture has been identified as an overriding climate factor in determining rates of respiration in a range of systems (Liu et al., 2009), this finding was, in general, expected. However, the finding of a strong sensitivity to moisture at the very lowest levels was more striking. Yuste et al. (2007) considered soil moisture effects in a savannah system, and, finding similar flux rates across a comparable range of moisture to our study, proposed a Boltzman sigmoid function fit to the data. Such a fit suggests no moisture response below  $\sim 18\%$  vol, yet across the same range, our data (total dataset range 8–55% moisture) show a very strong and significant response. This clearly highlights how mesic systems (as in the current study) have a lower resilience to such extremes when compared to the Mediterranean systems studied by Yuste et al. (2007). Indeed, the direction and magnitude of response soil moisture effects are strongly dependent on antecedent conditions



**Fig. 5.** Respiration from incubated soils across temperature ramp. Values are means  $\pm$  SEM, elevation of the four study sites is shown in metres above sea level.

**Table 3**

$Q_{10}$  values derived from incubated soils. Coefficients and significance values were derived from the fit of Eq. (1) (see text) to calculated flux data for the four study sites with elevation shown in metres above sea level.

Site	$R^2$	P	$Q_{10}$
1350 m a.s.l	0.87	0.006	1.76
1010 m a.s.l	0.85	0.009	1.55
570 m a.s.l	0.82	0.014	1.88
395 m a.s.l	0.95	0.001	1.73

and ecosystem type, as demonstrated by Sowerby et al. (2008). There, mesic systems became strongly water limited under simulated summer drought, yet respiration rates in hydric systems benefitted from moisture reduction. This emphasises the role of certain thresholds around moisture optima (Beier et al., 2012; Suseela et al., 2012), which can, not only directly constrain respiration, but can also constrain the relative strength of temperature as a driver (Lellei-Kovács et al., 2011).

In our study, such thresholds were addressed by our second hypothesis, and here we observed a strong effect, but further than expected, only the control site at 1350 m retained any significant sensitivity to temperature. This indicates that average moisture contents below the threshold of  $0.3 \text{ m}^{-3} \text{ m}^{-3}$  (despite occasional increases) were sufficient to remove the general sensitivity to temperature. This provides evidence of a moisture threshold between the conditions at the control site and the nearest transplantation site (1010 m). Interestingly, the control site did experience short periods of drought conditions, with moisture content falling to levels comparable to the mean values at transplantation sites. Despite this, the rate and temperature sensitivity were generally maintained, suggesting that it is the cumulative effect of drought that causes the change in system response, and that at higher elevation there is a greater degree of resilience to short periods of punctuated drought (Bérard et al., 2012; Evans and Wallenstein, 2011). Gavazov et al. (2013b) also suggested that a threshold of moisture control on plant community exists along the gradient, yet in the case of plant productivity, this was not observed until the site at 570 m. This is also a reflection of the different temporal scales involved in each process, but reinforces how mineralisation processes are exceptionally sensitive to change during the growing season, perhaps more so for the heterotrophic component of soil respiration (Scott-Denton et al., 2006).

The combination of vegetation and mineralisation process thresholds and sensitivity to change underpins the coupling of the autotrophic and heterotrophic components, and highlights the role of plant respiration in the soil respiration metric. In the work by Flanagan and Johnson (2005), soil moisture interacted with seasonal changes in plant biomass to explain 94% of variation in standardised ecosystem respiration, which likely encapsulates the autotrophic contribution to respiration, the indirect effect of exudate turnover, and the prevailing effect of soil moisture. In our study, whilst unable to compute a comparable biomass index, it is most likely that a large proportion of the variation among sites not explained by moisture or temperature would be driven by the observed reduction in biomass at transplantation sites.

#### 4.2. Temperature incubations

Following the observation of a strong in situ constraint by soil moisture, we expected that removal of drought stress would establish a temperature response in all soils under a controlled temperature ramp. We further expected that this sensitivity might vary such that sites that experienced greater in situ temperatures would have lower intrinsic temperature sensitivity. The cessation of drought conditions led to a temperature response in all soils, and contrary to our expectation, this sensitivity was comparable among sites. The derived  $Q_{10}$  values were similar to previous work under laboratory conditions (Yuste et al., 2007; Schindlbacher et al., 2010), and in the compartmentalisation exercise by Suseela et al. (2012). These findings suggest an intrinsic ability to rapidly respond to

more favourable conditions upon rewetting to field capacity, and to equally augment mineralisation rates as a function of temperature, despite the variability in antecedent conditions. The similarity in temperature response must then be an underlying trait of the microbial community, whereas the differences in flux magnitude (control site being much greater than transplantation sites) most likely reflect possible variation in the quantity of available substrate and the microbial biomass size.

Fierer and Schimel (2003) suggested that much of the respiration response seen after wetting of previously dried soil is derived from mineralisation of intracellular compounds as a response to osmotic stress, which might link magnitude of response to microbial biomass size. Yet whether this is due to accumulated osmolytes, or merely the respiration of microbial cellular machinery is unclear, especially as the former has recently been discounted by Boot et al. (2013). Wang et al. (2003) observed substrate availability indices to explain much of the rewetting response, and, despite their claim of less contribution from microbial biomass, still found a strong ( $r = 0.81$ ) correlation to respiration. Variation in the microbial community was not explicitly addressed in the current study, but samples from the same plots taken in spring (unpublished) show the control site (1350 m) to have significantly greater biomass C and N than sites at 1010 and 570 m (site 395 not sampled), adding some weight to the magnitude-biomass link. The similarity in 'intrinsic' temperature sensitivity (Davidson and Janssens, 2006) further suggests that the quality of the dominant substrates must remain broadly similar, as the temperature sensitivities of distinct pools of SOM (Farrar et al., 2012) may have become apparent if quality varied among sites. Despite some similarity to the type II acclimation described in Bradford et al. (2008), given the complete lack of significant difference in  $Q_{10}$ , we believe that this observation is not evidence of a thermal acclimation per se, and is more likely a response to moisture-induced effects on substrate and microbial biomass. A comparable intrinsic sensitivity among all sites indicates that the heterotrophic component of soil respiration is unlikely to have to access more recalcitrant (and temperature sensitive) organic matter over the short-term, under climate change, suggesting no significant functional changes. However, if productivity is reduced, cascade effects of reduced organic matter inputs to longer-lived pools may, in the long term, reduce soil C stocks (Knapp et al., 2002).

#### 4.3. Soil organic matter and chemistry

Changes in the cation content (mainly Ca) and in soil pH at drier and warmer transplantation sites suggest some fundamental alteration to base cycling. Dry/rewet events may have some contribution to this observation, especially if they result in disaggregation (Cosentino et al., 2006) and a general flushing of exchangeable cations. Reduction in base cycling from slower decomposition may also contribute, especially if important cations are retained in litter or light fraction organic matter. Direct effects of moisture are difficult to reconcile though, as evidence for reduced moisture leading to a drop in pH is confined in the literature to peats (Clark et al., 2006), following the established relationship between the oxidation of reduced sulphur and the production of protons. However, in mineral soils, to our knowledge, a moisture-induced drop in soil pH has not been observed, and whilst it is currently not possible to fully explain this observation, there are nevertheless likely to be consequences for soil function.

The hypotheses derived from lower plant productivity estimates suggest that reduced litter input should result in a lower quantity of organic matter existing as low-density material, as this material results from the initial stages of decomposition, and closely reflects litter input (Schulze et al., 2009). Contrary to our expectation, we found the proportion of  $\text{SOM}_{lf}$  and the content of C and N increased downslope, albeit only significantly when comparing the %N between the 1350 m and 395 m sites. This accumulation of  $\text{SOM}_{lf}$  suggests, perhaps irrespective of changes in litter input quantity, that the initial stages of



decomposition (especially physical fragmentation) are retarded in transplanted soils. Most likely this will be due to direct effects of lower moisture, but perhaps also to downwards migration of litter-degrading invertebrates (Briones et al., 2009). Such an 'isolation' of recently deposited material may represent a transient store of organic matter during the drier period, but one that is rapidly accessed under wetter conditions of longer duration, such as during winter. Unlike in previous work (Schindlbacher et al., 2010), the isolated SOM<sub>lf</sub> CN ratio did not explain any of the mineralisation data nor its temperature sensitivity. The fact that despite such large changes in function, we only observe small changes in the nature of SOM<sub>lf</sub>, and none in bulk SOM would suggest that decomposer communities may have a rapid recovery capacity during the post-growing-season phase which may dampen the effects of climate change over short time frames.

## 5. Conclusion

Climate manipulations of temperature and moisture along elevation gradients allow for estimation of combined effects on ecosystem processes, and here we observed a significant and overriding effect of moisture on soil respiration and its temperature response in a pasture system. The climate manipulations were shown to have affected the decomposer community response to temperature in terms of the magnitude of the flux, but not its intrinsic temperature sensitivity under standardised conditions. This highlights the resilience of the microbial community to extreme climatic change, but reinforces the reduction in the capacity to respond, and the consequences thereof for mineralisation processes and soil function. Initial observations on changes in the quantity and quality of the SOM<sub>lf</sub> indicate that accumulations of unprotected organic matter may lead to a greater C and N source for decomposer communities during the winter, therefore potentially shifting a large proportion of the annual C and N budget outside of the growing season.

The consequences of reduced function during the growing season carry negative implications for the sustainability of productivity in pasture systems, especially if limiting nutrients are only slowly returned to available pools. Whilst our results do not imply an increase in the losses of C as a function of climate change, a reduction in soil function will alter the storage capacity of C and macronutrients, which in the longer term will inevitably erode the stocks of C and N from organic matter. It is therefore pertinent that emphasis be made on understanding the seasonal dynamics of nutrient cycling in systems under climate change scenarios, especially those that exhibit reduction in soil function during the growing season. Further, there is a need to identify whether such changes feed back to community composition and productivity such to modify the sustainable use of sub-alpine pasture systems.

## Conflict of interest

I write to confirm that there are no conflicts of interest regarding my current submission.

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