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Published in:
Methods in ecology and evolution

DOI:
10.1111/2041-210X.12408

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Krab, E. J., Cornelissen, J. H. C., & Berg, M. P. (2015). A simple experimental set-up to disentangle the effects of altered temperature and moisture regimes on soil organisms. *Methods in ecology and evolution*, *6*(10), 1159-1168. https://doi.org/10.1111/2041-210X.12408

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Methods in Ecology and Evolution

British Ecological Society

Methods in Ecology and Evolution 2015, **6,** 1159–1168 doi: 10.1111/2041-210X.12408

A simple experimental set-up to disentangle the effects of altered temperature and moisture regimes on soil organisms

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Summary

1. Climate manipulation experiments in the field and laboratory incubations are common methods to study the impact of climate change on soils and their biota. However, both types of methods have drawbacks either on their mechanistic interpretation or ecological relevance.

2. We propose an experimental set-up that combines the best of both methods and can be easily obtained by modifying widely available Tullgren soil fauna extractors. This set-up creates or alters temperature and moisture gradients within intact field soil cores, after which soil biota, their activity and vertical movements can be studied. We assessed the performance and demonstrated the applicability of this set-up through a case study on Collembola response to changes in microclimatic gradients in peat bogs.

3. Warming created a vertical temperature gradient of 14°C in peat cores without varying soil moisture conditions, while at a given temperature regime, precipitation and drought treatments shifted natural soil moisture gradients to 'wetter' and 'drier', respectively. This allowed for disentangling interacting warming and moisture effects on soil fauna. In our case study, Collembola communities showed peat layer-specific responses to these climate treatments. Warming decreased Collembola density and altered community composition in the shallowest layer, whereas precipitation increase affected Collembola community composition in the deepest layer.

4. We showed that climate change can have layer-specific effects on soil organisms that are 'hidden' by not taking microclimatic vertical gradients into account. This experimental set-up facilitates studying (multitrophic) organism responses to climate changes, with only a small adjustment of equipment that is often already present in soil ecology laboratories. Moreover, this set-up can be easily customized to study many more other research questions related to wide-ranging organisms and ecosystems.

Key-words: Climate change, Collembola, food web, laboratory, peat moss, soil cores, spatial vertical distribution

Introduction

Although field climate manipulation experiments may give us a realistic view of ecosystem responses to climatic changes, these responses are usually context dependent. Often, the underlying mechanisms remain unclear, partly because they remain hidden in a 'black box' without destructive sampling and partly because different drivers and different processes may all interact. To be able to comprehend the mechanisms by which climatic changes impact on soils, its biota and their functions, standardized experiments are often carried out using (homogenized) soil or litter incubations in micro- or mesocosms under laboratory conditions (e.g. Lavoie, Mack & Schuur 2011; A'Bear, Boddy & Jones 2012). Though the relative simplicity of these laboratory incubations increases our understanding of the mechanisms underlying responses to climate change, they might also oversimplify them, resulting in a discrepancy between laboratory and field observations.

As an alternative to using climate-controlled artificial systems that contain a standardized soil and/or a specific (group of) model soil organisms, the use of terrestrial model ecosystems (TME) has been proposed (Morgan & Knacker 1994; Knacker *et al.* 2004; Foerster *et al.* 2009). For these TMEs, intact soil cores including living vegetation are excavated from the field, placed in mesocosms and incubated in the field or laboratory. In this way, soil organisms are incubated in their own substrate in an ecosystem comprising multiple trophic levels and an intact soil structure that contains gradients in SOM quality and porosity. When placed in the laboratory, soil temperature and moisture conditions can be controlled or manipulated (Kools *et al.* 2008).

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A factor that is frequently overlooked in laboratory assessment of responses of soil organisms to climate is the natural occurrence, and especially co-occurrence, of vertical temperature and moisture gradients in soils. These microclimatic gradients occur naturally in manipulation experiments in the field (Krab *et al*. 2010, 2013), but are often absent in laboratory incubations in climate rooms. In this way, the spatial distribution of organisms or their behavioural responses to climatic changes are not accounted for as all organisms get exposed to more or less the same microclimatic conditions. For example, Collembola (springtails) in field soils show a strong vertical patterning in community composition (Berg *et al*. 1998). Species that generally live deeper down the soil profile might show a high sensitivity to warming (Van Dooremalen, Berg & Ellers 2013) but hardly respond to a manipulated increase in air temperature in the field (Krab *et al*. 2013), since the deeper soil layers into which these mobile animals can 'flee' are buffered from increased air temperatures. These changes in vertical patterning of soil organisms might have large impacts on ecosystem processes (Faber 1991; Briones, Ostle & Garnett 2007) but are easily missed in manipulation experiments when sampled in a non-stratified way. This might explain the fact that in field climate manipulation experiments, often only subtle responses of the soil invertebrate community are observed (e.g. Coulson *et al*. 1996; Sohlenius & Bostrom 1999; Sjursen, Michelsen & Jonasson 2005). Hence, the spatial distribution of soil organisms and their ability to change this distribution might be a key determinant of the impact of climatic changes on soil organism-mediated ecosystem processes (Ettema & Wardle 2002), but this aspect is often largely removed from laboratory experiments performed in controlled and standardized climate rooms.

To study soil organism responses to climatic changes by taking into account vertical gradients in soil structure, biotic players and microclimate in a controlled laboratory set-up, we propose a novel modification of the Tullgren soil fauna extraction set-up (Tullgren 1918) that is already available and has been widely used for invertebrate animal extractions in numerous laboratories across the world. Our simple approach allows for testing both the separate effects and interactions of soil temperature and moisture on soil organisms without the need for costly laboratory facilities such as climate-controlled greenhouses or incubators. The vertical gradients in temperature and moisture conditions that this method creates within the soil cores will test the effects of climate change on (vertical distribution of) soil organisms in a controlled but more realistic setting than by using common laboratory incubations.

In brief, our approach entails excavating intact soil cores from the field and placing these in semi-closed containers (TMEs) that allow for water and gas exchange. These containers are subsequently placed in a modified Tullgren extractor, which in its unmodified form is commonly used for drought/heat extraction of soil invertebrates (Van Straalen & Rijninks 1982). In this set-up, it is possible to manipulate microclimate using a heat source, water addition and silica grain application. The Tullgren heat source above the cores simulates temperature increase (increased evapotranspiration is compensated for by adding water), while supplementary water addition mimics precipitation increases. Silica grains placed above the soil core decrease air humidity and initiate drought. By using these treatments in a factorial design, it is not only possible to separate temperature from inundation and drought effects, but also to test for scenarios in which multiple climate factors will change simultaneously or in sequence. Moreover, this set-up allows assessment of the impact of changes in climatic drivers on the vertical distribution of soil biota when microclimatic manipulation is combined with a spatially explicit destructive sampling design.

In this study, we will show that this simple set-up, which creates planned vertical gradients both in soil temperature and moisture in soil cores, goes a long way towards a controlled system to unravel the mechanisms behind field-relevant responses of soil organisms to climatic changes. We will demonstrate this set-up by presenting detailed methods and results from a case study in which we test how Collembola, a particularly abundant and diverse group of soil invertebrates in (sub)arctic peatlands (Woodin & Marquis 1997), respond to different gradients in soil temperature and moisture in peat bogs dominated by *Sphagnum* moss. We hypothesize that changes in the vertical gradient of soil temperature and moisture will affect the overall vertical distribution pattern of Collembola (rather than their total density). Additionally, we expect individual Collembola species to respond differently to altered vertical soil temperature and moisture gradients initiated by temperature increase, drought and water addition. Therefore, we expect a layer-specific change in community composition due to the climate treatments. We will place the results obtained from this case study into a broader ecological context and discuss the strengths and weaknesses of this set-up, after which we will give recommendations for multiple further applications.

Methods

COLLECTION OF SOIL CORES

Soil cores (ø 10 cm, height 12 cm) were carefully excavated with a soil corer from a blanket peat bog on a slightly sloping bank of Lake Torneträsk near Abisko Research Station in subarctic Sweden (68°21°N, 18°49°E, 340 m above mean sea level) in July 2011. The peat moss *Sphagnum fuscum* (Schimp.) H. Klinggr., dominated the vegetation in this peat bog, growing intermingled with vascular plants (with a cover of about 25%) mainly consisting of *Empetrum hermaphroditum* Hagerup, *Betula nana* L., *Vaccinium microcarpum* L., *Vaccinium uliginosum* L., *Rubus chamaemorus* L., *Eriophorum vaginatum* L. and *Calamagrostis lapponica* (Wahlenb.) Hartm. (Aerts *et al*. 2009). The soil cores, 48 in total, were excavated blockwise (8 blocks, 6 cores each) from the field site and directly placed in PVC containers (ø 10 cm, height 13 cm), packed in plastic bags and transported into the laboratory of Abisko Research Station. There, the PVC containers were weighed and stored overnight at 5°C before placing them in the modified Tullgren set-up.

A MODIFIED TULLGREN SET-UP TO DISENTANGLE THE EFFECTS OF MOISTURE AND TEMPERATURE ON SOIL ORGANISMS

Four Tullgren extractor sets (Burkard Scientific, Uxbridge, Middlesex, UK) were used for this set-up (Fig. 1). Each of these Tullgren sets consisted of 2 rows of 6 extraction places each (which corresponded to two experimental blocks per set), and the sets were placed in a laboratory with a relatively constant air temperature (20–23°C).

The PVC containers containing the soil cores were placed in these sets. The containers were closed at the bottom by a removable lid with a small sealable hole (ø 0·2 mm) to regulate the water content during the experiment. They were closed at the top with a fine mesh (60 μm) that left approximately 1 cm of air space between the peat core and the top of the container. This mesh prevented the soil invertebrates from escaping while allowing for gas exchange. The PVC containers consisted of three stacked rings of 3 cm height each and one top ring of 4 cm, taped to each other water and airtight. These measures would facilitate fast slicing of the peat core with a knife at harvest to prevent movement of Collembola between soil layers, and to enable subsequent safe transportation of each slice for extraction and analysis. Before that, these containers were exposed to warming, moistening, desiccation and all its combinations for a period of 21 days (Table 1).

The Tullgren sets were modified to construct a factorial design in which the effects of warming and three different moisture regimes, and each of their combinations, on Collembola communities could be tested throughout the vertical peat profile sampled (Table 1). This resulted in 6 treatments, which were replicated 6 times. Each Tullgren row (block, see above) of 6 extraction places contained all treatments in a random distribution. To apply the warming treatments, 25-W light bulbs placed 25 cm above the containers were used. These bulbs were placed in the top of metal cylinders that guided the heat down towards the soil cores, without spilling heat to the neighbouring containers (Fig. 1). In the ambient warming treatments, the cylinder and light bulb were removed.

By using a 25-W light bulb as a heat source, this Tullgren set created a 14°C temperature gradient throughout the soil cores that were placed in each extraction place. Within-core temperatures were relatively high (*c*. 20–25°C, Fig. 2a) but lay within the range of soil core temperatures in the field (Krab *et al*. 2013). Maximum surface temperatures (Fig. 2b) resembled high but realistic summer soil surface temperatures in the field (Westermann, Langer & Boike 2011) that are likely to become more common under near-future subarctic climate warming (Krab *et al*. 2013).

The moisture treatments were established by altering the (ambient) soil moisture content of the soil cores. In the ambient soil moisture treatments, a water content of 86% (percentage of sample weight that was comprised by water), which was the average water content of the cores taken from the field, was maintained throughout the experiment. In the water addition treatments, a rain event was simulated by adding demiwater (which is broadly representative of rainwater in this area) to the top of the cores, to bring the core to a water content of 88%. The average amount of added water per core was 71 ml (exact amount was dependent on the initial core weight).

Silica grains were used to simulate desiccated conditions for the drought treatments. Two millimetres above the fine mesh on top of the soil container a plastic Petri dish (ø 9 cm) filled with silica gel grains (24 g dry grains) was placed. The top of the Petri dish was covered with a coarse mesh (3 mm) to keep the grains in and was placed upside down on the top of the container with the exposed grains towards the soil cores' surface. To control for possible effects of silica grain application, all treatments received a Petri dish with silica grains; however, only in the drought treatment, the silica grains were dehydrated in a stove and replaced by new dried grains daily (Fig. 1). By doing so, the relative air

Fig. 1. (a) Schematic drawing, (b) a detail picture and (c) an overview picture of the experimental set-up. Each of the containers contained a *Sphagnum fuscum* core and had a water inlet at the bottom and a fine mesh on top. On top of each container, a Petri dish filled with silica grains was placed, with the grains facing the core's surface. Above each of the warmed containers, a 25-W light bulb was placed in a metal tube guiding the heat down towards the soil core surface.

Table 1. Factorial design; treatments were created by modifications to the Tullgren set-up as explained in the legend

	Warming treatment	
	Ambient	Temperature increase
Soil moisture treatment		
Drought	AD	TD
Ambient	AA	TA
Water addition	AW	TW

AD, Ambient temperature, drought from the top using desiccated silica grains. Soil moisture gradient: no temperature gradient. Start the experiment by drying out the core to the desired percentage (83%).
AA, Ambient temperature, ambient soil moisture conditions (86%). Soil moisture gradient: no temperature gradient.
AW, Ambient temperature, water addition. Soil moisture gradient: no temperature gradient. Start the experiment by adding demiwater at the top and bring moisture content to desired percentage (88%).
TD, Temperature increase and drought treatment. Temperature gradient and soil moisture gradient. Start the experiment by drying out the core to the desired percentage (83%), desiccation from the top using desiccated silica grains.
TA, Temperature increase only. Temperature gradient and ambient soil moisture gradient.
TW, Temperature increase and water addition. Soil moisture gradient and temperature gradient. Start the experiment by adding demiwater at the top to bring moisture content to desired percentage (88%).
All treatments: Replace respired water daily by adding (natural peat) water through the bottom so that moisture percentage will stay more or less constant in time.

humidity above the soil core was decreased in the drought treatment; this desiccated the cores to a water content of 83%.

The chosen water contents in our moisture treatments were realistic 'extreme' moisture conditions that can be found in the field (Krab *et al.* 2013). In all treatments, the evaporated water was replaced daily by 'groundwater' taken from a small rainwater-filled pond in the peat bog, to maintain constant soil moisture conditions throughout the experiment. This water was added at laboratory temperature, to the deepest layer of the soil core, by injecting it through a small hole in the bottom of the soil container. The water was assumed to naturally redistribute through the core by capillary action.

CLIMATE CONTROLS

To follow the moisture content and the temperature gradient in the soil cores during the experiment without disturbing the soil fauna, we added two climate monitoring blocks to the set-up, resulting in a total of 8 randomized blocks (6 + 2 blocks). Each of these climate-monitoring blocks received all the climate manipulation treatments.

In one of the two climate-monitoring blocks, we measured temperature (to the nearest 0·1°C) continuously by placing sensors (12-Bit Temp Smart Sensor - S-TMB-M006; Onset, Bourne, MA, USA) at 3, 6 and 9 cm depth in the soil cores from the start until the end of the experiment. Due to the limited availability of temperature sensors, we rotated the sensors daily between cores within this block during the experiment. This resulted in measurements for each treatment core for at least 3 days (3 × 24 h) during the first 2 weeks of the experiment. In the other block, we measured temperature at 3, 6 and 9 cm depth in the soil cores daily (to the nearest 0·1°C) with a hand-held soil thermometer for 6 consecutive days during the start of the experiment (a single measurement per day at midday). After these 6 days, we added (extra)temperature sensors (12-Bit Temp Smart Sensor - S-TMB-M006; Onset) that logged temperatures for 4 more consecutive days at 3, 6 and 9 cm depth in the soil cores in the warming treatments (and at a single depth in the ambient warming treatments). As measurement days overlapped for the two blocks, we were able to pair measurements to compare temperature profiles between these blocks.

After 14 days (7 days before final harvest), we harvested the cores of these two climate control blocks and measured soil moisture content gravimetrically (data not presented). After 21 days, the 6 blocks of the core experiment were harvested: Surface temperature was measured with a hand-held soil thermometer to the nearest 0·1°C, and volumetric water content of the layers was calculated by weighing the soil core slices directly and after drying the cores for 48 h, at 70°C after Tullgren fauna extraction.

HARVEST

After running these climate regimes for 21 days, the soil cores were sliced in four layers of 3 cm each (see taping treatment above): (i) the top layer, consisting of living moss capitulum mixed with fresh moss litter, (ii) the second layer, consisting of new and coarse fragmented moss litter, (iii) the third layer, consisting of more fragmented moss litter, and (iv) the deepest layer, comprising fine fragmented moss litter mixed with older peat particles. The slices, each still contained by a PVC ring to prevent compaction, were sealed into plastic bags with sufficient air, kept cooled (4°C) and transported to VU University, Amsterdam for soil fauna extraction within 48 h. Soil fauna was extracted from each separate soil core slice by a conventional Tullgren Funnel fauna extractor (Van Straalen & Rijninks 1982). Collembola were extracted in 70% ethanol and identified up to species level (using Fjellberg 1998, 2007), and species abundances were counted separately for each soil layer.

STATISTICS AND CALCULATIONS

The effects of the warming and moisture treatments on soil temperatures were tested during the experiment in the two climate-monitoring blocks. The effects of measurement day (6 days) and depth in the soil on soil temperatures were tested using two-way repeated-measures ANOVA. To test for block effects in the climate control blocks, we tested for differences between temperatures at different depths using a pairwise *t*-test. To calculate average soil temperatures at depths 3, 6 and 9 cm in the soil cores, only measurements taken in both blocks on the same day were included. To test for differences in depth-specific temperature between the warming and the control treatment, an additional one-way ANCOVA, with depth as a covariate and temperature treatment as a fixed factor, was carried out (assuming no effect of moisture treatment on soil core temperatures). For this analysis, average depth-specific temperatures were calculated for the two climate blocks, and temperatures of all warmed soil cores were considered replications of the temperature treatment while all non-warmed cores were considered replicates of the control (3 cores per climate monitoring block, $n = 6$). The effects of temperature and moisture treatments on surface temperatures were tested using a two-way factorial ANOVA. The effect of the climate treatments on soil moisture content at time of harvest was analysed using a two-way factorial ANCOVA in which temperature and moisture treatments were fixed factors and depth was included as a covariate.

To test the effects of the temperature and moisture treatments on total Collembola density per soil core (no separation in layers), we performed a two-way factorial ANOVA. Changes in Collembola vertical (density) distribution due to our treatments were tested by two-way

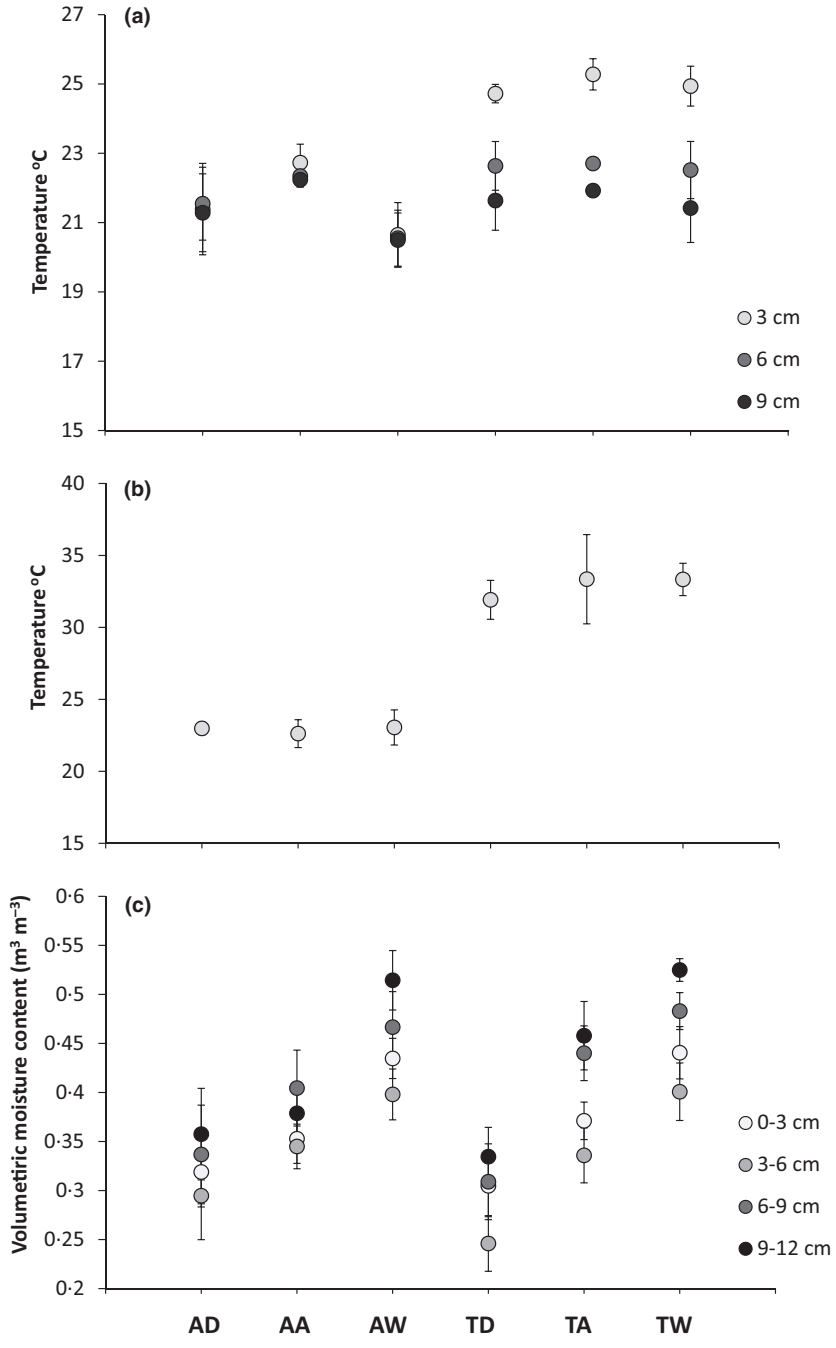

Fig. 2. (a) Average soil temperatures, (b) soil surface temperatures and (c) volumetric soil moisture content. Soil temperatures were measured in climate monitoring blocks (n = 2) and measured at 3 cm deep (light grey circles), 6 cm deep (grey circles) and 9 cm deep (dark grey circles). Soil moisture content was measured gravimetrically from four soil layers: 0–3 cm (white circles), 3–6 cm (light grey circles), 6–9 cm (grey circles) and 9–12 cm (dark grey circles). Soil temperatures were measured at midday during the experiment. Soil moisture content and surface temperatures were measured at day of harvest for Collembola extractions (n = 6). Warming treatments were ambient (A) and temperature increase (T). Soil moisture treatments were drought (D), ambient (A) and water addition (W). Treatments are always a factorial combination of warming and soil moisture manipulation.

factorial ANCOVA on log-transformed data, in which temperature and moisture treatments were fixed factors and soil layer (0–3 cm, 3–6 cm, 6–9 cm, or 9–12 cm deep) was included as a covariate. To test whether Collembola community composition was affected by the climate treatments (and thus for dissimilarities in species composition between communities found in the different cores or soil layers), we performed a permutation test for multifactorial multivariate analysis of variance (two-way factorial PERMANOVA, distance matrix: Bray-Curtis, no. of permutations: 999) (Anderson 2001). The test statistic is a multivariate analogue to Fisher's F-ratio and is calculated directly from a Bray–Curtis similarity matrix; P-values are then obtained using permutations. We performed this test both on the community as a whole and for layer-specific communities (separate tests per layer). We performed this test on untransformed data, as this test is 'distribution free' because it relies on permutation procedures to obtain P-values (Anderson 2001). All statistical analyses were carried out using R (version 3.1.2; R Foundation for Statistical Computing, Vienna, Austria) in which the PERMANOVA test is available in the 'vegan' package.

Results

SOIL TEMPERATURE

Soil temperatures were significantly different between measurement days ($F_{5,25}$ = 410·7, P > 0·001). Soil temperatures at

different depths varied ($F_{5,66} = 93{\cdot}2$, $P < 0{\cdot}001$), and decreased with depth most likely caused by the effects of the temperature treatment (Fig. 2a). There was no significant difference between soil temperatures at different depths of similar treatments between the two climate-monitoring blocks ($P < 0{\cdot}001$) (Table 2). Moisture treatment did not seem to affect soil temperatures [Fig. 2a; this could not statistically be tested due to low replication, but we consider the slightly warmer soil temperatures of the non-warmed ambient moisture treatment (AA) to be a result of low replication numbers ($n = 2$)]; therefore, we proceeded with an ANCOVA testing effects of warming, whereby we assumed no effect of moisture treatment on soil temperatures. Warming treatment affected soil temperatures considerably ($F_{1,32} = 20{\cdot}8$, $P < 0{\cdot}001$), and there was a significant interaction between soil temperature at a specific depth and warming treatment ($F_{1,32} = 8{\cdot}0$, $P < 0{\cdot}01$), indicating that soil temperatures at different depths differed between the warming treatments. We attribute this to the fact that there was no temperature gradient in non-warmed cores, whereas there was a temperature gradient in the warmed cores. Surface temperatures at harvest (Fig. 2b) showed a clear effect of warming treatment ($F_{1,30} = 420{\cdot}3$, $P < 0{\cdot}001$) and the absence of an effect of moisture treatment on surface temperatures. The surface temperature patterns broadly corresponded between the climate-monitoring blocks and the harvested cores (Fig. 2a vs. b).

SOIL MOISTURE

The soil moisture treatment had a significant effect on moisture regime ($F_{2,32} = 3{\cdot}7$, $P < 0{\cdot}05$) which differed between soil layers at different depths [($F_{1,32} = 19{\cdot}7$, $P < 0{\cdot}001$); Fig. 2c]. Deeper layers were generally moister than more shallow layers, except for surface layers, which were slightly moister than the consecutive layer. Further, the temperature treatment did not affect the soil moisture. There was no interaction between soil moisture treatment and soil moisture at different depths, indicating that the vertical soil moisture profile did not significantly change due to the moisture treatments.

Table 2. Overview of the used statistical analyses and their output

	Test for	Test type		d.f., residuals	*F*	*P*
Abiotics						
Soil temperature	Measurement day and layer	Two-way repeated-measures ANOVA	*Layer*	5,25	93·22	<0·001
			Day	5,66	410·7	<0·001
	Block effects	Pairwise *t*-test				<0·001
	Layer-specific temperatures	One-way ANCOVA	*Temperature*	1,32	20·84	<0·001
			Layer	2,32	0·05	ns
			TxL interaction	1,32	8	<0·01
	Surface temperatures per treatment	Two-way factorial ANOVA	*Temperature*	1,30	420·34	<0·001
			Soil moisture	2,30	0·56	ns
Soil moisture	Treatment effects per layer	Two-way factorial ANCOVA	*Temperature*	1,132	0·86	ns
			Soil moisture	2,132	3·74	<0·05
			Layer	1,132	19·67	<0·001
Collembola						
Whole core	Density, treatment effect	Two-way factorial ANOVA	*Temperature*	1,30	1·51	ns
			Soil moisture	2,30	1·17	ns
	Community composition, treatment effect	Two-way factorial PERMANOVA	*Temperature*	1,30	1·67	ns
			Soil moisture	2,30	0·72	ns
Layer specific	Density, treatment effect layer specific	Two-way factorial ANCOVA	*Temperature*	1,132	5·05	<0·05
			Soil moisture	2,132	0·95	ns
			Layer	1,132	0·42	ns
			TxL interaction	1,132	3·49	<0·1
	Community composition, treatment effect (separate test per layer)	Two-way factorial PERMANOVA (0–3 cm)	*Temperature*	1,30	3·67	<0·01
			Soil moisture	2,30	0·74	ns
		Two-way factorial PERMANOVA (3–6 cm)	*Temperature*	1,30	0·67	ns
			Soil moisture	2,30	0·71	ns
		Two-way factorial PERMANOVA (6–9 cm)	*Temperature*	1,30	0·56	ns
			Soil moisture	2,30	0·51	ns
		Two-way factorial PERMANOVA (9–12 cm)	*Temperature*	1,30	1·83	ns
			Soil moisture	2,30	3·61	<0·01

Only significant (up to $P < 0{\cdot}1$) interactions are included.

COLLEMBOLA DENSITY AND COMMUNITY COMPOSITION

Although there seemed to be a slight decline in overall Collembola density in the climate manipulation treatments relative to the ambient soil moisture and temperature (AA) treatment (Fig. 3), this decline was not statistically significant (Table 2). The overall Collembola density in the ambient treatment was 615 ± 244 individuals dm^{-3}. Collembola community composition of the soil core as a whole did not change in response to any of our treatments. However, when including layer-specific changes in density in the statistical analysis, we found a statistically significant decline in Collembola density due to the warming treatment ($F_{1,132}$ = 5·05, P < 0·05) and a weak interaction between warming and soil layer, suggesting that warming changed density distribution over soil layers ($F_{1,132}$ = 3·49, P = 0·06) (Table 2).

Warming also significantly affected species composition of the top layer (0–3 cm) ($F_{1,30}$ = 3·7, P < 0·05), whereas the soil moisture treatment affected species composition in the deepest layer (9–12 cm) ($F_{1,30}$ = 3·6, P < 0·05). Both of these community shifts are most likely caused by a decline in density of *Folsomia quadrioculata*, the predominant Collembola species in this northern peatland. However, the apparent decline in *F. quadrioculata* in these layers due to the treatments did not seem to correspond to a consistent increase in density in other soil core layers undergoing the same treatment.

Discussion

TULLGREN SET-UP SUCCESSFULLY MANIPULATES VERTICAL MOISTURE AND TEMPERATURE GRADIENTS

Our experimental set-up successfully created and altered temperature and moisture gradients. There was a natural gradient in moisture conditions in all treatments. The precipitation and drought treatments were either shifted towards 'wetter' or 'drier' than the ambient treatment, and similar in all treatments, with deeper layers having higher soil moisture content whereas shallower layers were drier. The manipulated soil moisture gradient corresponded to gradients created in the field by a precipitation manipulation experiment carried out simultaneously with this study (Krab *et al.* 2013). Different from what we expected, top layers were not always driest as in ambient conditions in the field (Krab *et al.* 2010, 2013). We attribute this to a slight underestimation of the volume of the top layer. Core slices were 3 cm thick, but the top PVC ring was 4 cm high to leave one cm of headspace. The top soil layer might therefore have been thicker than the other layers, and by expressing soil moisture by 'water per volume', this probably resulted in an overestimation of soil moisture content. Indeed, when considering a 4-cm-thick top soil layer in our calculations, the top layer would be consistently drier than deeper layers (data not presented). Alternatively, the water content of living *S. fuscum* capitulum might be higher than that of partly decomposed structures deeper down in the soil.

Warming created a temperature gradient of 14°C (21–35°C) from the surface of the soil core towards the deepest soil layer. Although our warming scenario was rather extreme, soil surface temperatures well above 25°C are not uncommon on summer days with high solar radiation in subarctic areas (Westermann, Langer & Boike 2011; Krab *et al.* 2013) and may become more frequent in the near future (Hansen, Satoa & Ruedy 2012). However, in a field situation, deeper layers are generally considerably cooler (average temperature at 9–12 cm depth around 10°C in July) (Krab *et al.* 2013), and temperature gradients from the soil surface to 12 cm depth are therefore steeper. As in the ambient temperature treatment no temperature gradient existed, we suggest that the addition of a

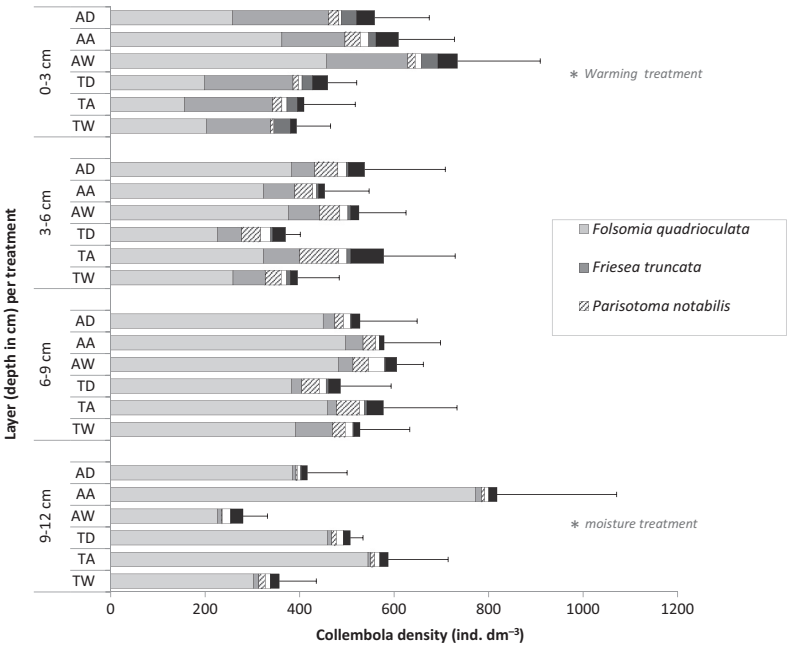

Fig. 3. Collembola density and community composition grouped by soil (depth) layer for each warming and soil moisture treatment. Warming treatments were: ambient (A) and temperature increase (T). Soil moisture treatments were drought (D), ambient (A) and water addition (W). Treatments are always a combination of warming and soil moisture treatment. Collembola density is indicated by bar length, and total density is the cumulative of species densities. Different shades of grey indicate different species; only the five most abundant species have been indicated in the figure. Error bars are standard errors (n = 6).

simple cooling system would improve the ability to translate and compare the obtained results to field situations.

Effects of warming on soil organisms are often attributed to their effects on soil moisture conditions (e.g. Makkonen *et al.* 2011). However, we showed that the effects of warming on Collembola community composition were significantly different from that of desiccation alone, even though the soil moisture regimes in the top layer were comparable between treatments. This indicates that warming does not only act on soil organisms by creating drought, but also that higher temperatures have a stand-alone effect. Thus, this set-up successfully separated moisture from temperature gradients and showed that soil organisms (at least Collembola) respond differently to their separate effects. So what exactly did our methodology enable us to find out about this?

COLLEMBOLA RESPONSES TO CHANGES IN MICROCLIMATE IN SOIL CORES

Collembola species composition of a deeper soil layer changed significantly due to a simulated increase in precipitation, whereas this shift in community composition could not be observed when considering the whole soil core. Likewise, community composition in the top layer of soil cores subjected to warming changed considerably (and its density declined slightly). However, when spatial distribution was not taken into account, no responses of the Collembola community could be detected, even though Collembola are known to show strong physiological responses to the manipulation of abiotic conditions such as heating, drought and waterlogging (e.g. Holmstrup, Hedlund & Boriss 2002; Kærsgaard *et al.* 2004; Van Dooremalen, Berg & Ellers 2013).

Collembola mortality during the experiment was low, and the average Collembola density in the ambient treatment (615 ± 244 ind. dm^{-3}) at day of harvest was somewhat lower than field density (730 ± 93 ind. dm^{-3}) (Krab *et al.* 2013), but was higher than in *S. fuscum* cores from a nearby similar field site (222 ± 39 ind. dm^{-3}; Krab *et al.* 2014).

Both of the observed layer-specific community shifts were caused by a decline in density of *F. quadrioculata*, which is the most dominant Collembola species in this northern peatland and known to respond to climatic changes in field manipulation experiments (Hertzberg & Leinaas 1998; Krab *et al.* 2013). The decline in *F. quadrioculata* in the top soil layer due to warming corresponded to the decline in *F. quadrioculata* density due to warming by a 'greenhouse' in the field experiment that was carried out simultaneously with this experiment (Krab *et al.* 2013). This field experiment did not show a statistically significant decline in *F. quadrioculata* in deeper soil layers in its precipitation manipulation experiment. However, the increased soil moisture conditions we created in our laboratory set-up were wetter than those that were created in the field study [$0{\cdot}51 \pm 0{\cdot}07$ m^3 H_2O m^{-3}, vs. $0{\cdot}40 \pm 0{\cdot}06$ m^3 H_2O m^{-3} (Krab *et al.* 2013)]. Similar to what we observed in our laboratory set-up, a warming-induced decline in *F. quadrioculata* did not correspond to an increase in density in other soil layers in the field experiment (Krab *et al.* 2013). However, this might be a short-term effect since the short duration of our (and the field) experiment did not include effects on reproduction.

Climate change can lead to changes in (vertical) density distribution and layer-specific shifts in community composition and suggests that different Collembola species indeed differ in sensitivity to the extreme abiotic stressors (Krab *et al.* 2010; Van Dooremalen, Berg & Ellers 2013). The extent to which these changes translate to changes in ecosystem processes such as decomposition and respiration is still unknown. Although soil invertebrate species composition can affect ecosystem processes significantly (Cragg & Bardgett 2001; Heemsbergen *et al.* 2004), it has also been suggested that soil invertebrate species are redundant (Laakso & Setala 1999; Bradford *et al.* 2002). Either way, shifts in spatial (vertical) distribution might have profound effects on ecosystem processes since decomposers have different net effects on carbon and nitrogen cycling depending on the soil layer in which they are active (Faber 1991; Berg 2000). Climate change manipulation experiments have shown the effects of climate warming on layer-specific carbon cycling (Dorrepaal *et al.* 2009), but only a few linked this to soil invertebrate activity (Briones, Ostle & Garnett 2007).

RECOMMENDATIONS AND MULTIPLE FURTHER APPLICATIONS OF THE SET-UP

Even though our set-up lacked a 'natural' temperature gradient in the ambient treatment, the responses of the Collembola community to the created temperature gradient in the warming treatment corresponded largely to observations in the field (Krab *et al.* 2013). Furthermore, the absence of a temperature gradient in the control helped to answer mechanistic questions about the importance of abiotic gradients for vertical distributions of soil biota. However, for testing other hypotheses, or to apply this method simultaneously with field experiments, it might be necessary to mimic realistic field conditions. To do so, the set-up could be either placed in a climate-controlled laboratory with the possibility to create local differences in temperature (see Kools *et al.* 2008) or a high-gradient Tullgren extractor can be used, where a thermostat regulates the temperature at both sides of the gradient (see Van Straalen & Rijninks 1982). Alternatively, cores could be cooled using (gel) ice packs or the set-up could be transferred outside, where the containers can be placed in cavities in the local soil. In the latter case, the natural local soil temperature gradient could be further manipulated by using the top part of the Tullgren set-up. Although these adaptations would somewhat reduce the simplicity of the initially proposed set-up, as more laboratory infrastructure will be necessary, it will benefit the extrapolation of the obtained results into a field ecological context.

Evidently, this set-up does not only limit itself to studying Collembola in peat soils, as many other types of soil and soil biota can be studied. Apart from applying it to other groups of soil fauna in wide-ranging ecosystems, it should be very well possible to study the response of the microbial commu-

nity, its activity and changes in various ecosystem properties at different soil depths. Moreover, it is possible to study the response of various trophic levels simultaneously as well as climate-induced trophic cascading (Lensing & Wise 2006). Since the soil system is closed for 'larger' soil biota, the addition and removal of certain trophic levels (e.g. by removing plants or adding invertebrate predators) can also be manipulated.

Conclusion

This experimental set-up creates, with only little adjustment of equipment that is present in most soil ecology laboratories, a system that facilitates studying (multi-trophic) biotic responses to changes in soil microclimate. This set-up allowed us to successfully disentangle the effects of soil moisture and temperature on Collembola community composition and distribution in a peat soil. It showed that exposure to (strong) warming and manipulation of moisture conditions can lead to changes in density and layer-specific shifts in community composition that in general seem to correspond with field observations. Due to its simplicity and relatively low cost (even when newly built), this set-up could easily be applied to the responses of different invertebrate and microbial communities to (changing) temperature and moisture regimes in different biomes. It could be stationed near remote field sites with limited facilities to be run alongside field manipulation experiments and used for teaching purposes. In brief, it represents a highly flexible, versatile and widely applicable approach, combining the strong points of a 'realistic' field experiment with those of an 'understandable' laboratory incubation.

Acknowledgements

We would like to thank the Abisko Scientific Research Station providing research facilities and hospitality. This study was funded by NWO (Netherlands Organization for Scientific Research), specifically by its International Polar Year program (ENVISNAR project) through Grant 851.40.060

Data accessibility

All data used in this publication are available online from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.6r6pn.

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Received 16 February 2015; accepted 6 May 2015
Handling Editor: Robert Freckleton