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Accepted Version

Milcu, A., Paul, S. and Lukac, M. ORCID:
<https://orcid.org/0000-0002-8535-6334> (2011) Belowground interactive effects of elevated CO₂, plant diversity and earthworms in grassland microcosms. *Basic and Applied Ecology*, 12 (7). pp. 600-608. ISSN 1439-1791 doi: 10.1016/j.baae.2011.08.004 Available at <https://centaur.reading.ac.uk/21815/>

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To link to this article DOI: <http://dx.doi.org/10.1016/j.baae.2011.08.004>

Publisher: Elsevier

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Type of contribution: **Research article**

Number of text pages: 25

Number of tables: 1

Number of figures: 2

Number of words including references, tables and figure captions: 5348

Appendixes: 1

**Belowground interactive effects of elevated CO₂, plant diversity and
earthworms in model grasslands**

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ABSTRACT

The potential interactive effects of future atmospheric CO₂ concentrations and plant diversity loss on the functioning of belowground systems are still poorly understood. Using a microcosm greenhouse approach with assembled grassland plant communities of different diversity (1, 4 and 8 species), we explored the interactive effects between plant species richness and elevated CO₂ (ambient and + 200 p.p.m.v. CO₂) on earthworms and microbial biomass. We hypothesised that the beneficial effect of increasing plant species richness on earthworm performance and microbial biomass will be modified by elevated CO₂ through impacts on belowground organic matter inputs, soil water availability and nitrogen availability. We found higher earthworm biomass in eight species mixtures under elevated CO₂, and higher microbial biomass under elevated CO₂ in four and eight species mixtures if earthworms were present. The results suggest that plant driven changes in belowground organic matter inputs, soil water availability and nitrogen availability explain the interactive effects of CO₂ and plant diversity on the belowground compartment. The interacting mechanisms by which elevated CO₂ modified the impact of plant diversity on earthworms and microorganisms are discussed.

Keywords: Climate change; Species richness; Above-belowground interactions; Water availability; Soil N; *Lumbricus terrestris*

49 **Introduction**

50 Understanding of the relationship between biodiversity, climate change and the ecosystem
51 services upon which we are heavily reliant emerged as a critical issue in the face of
52 increasing human-induced environmental change (Millennium Ecosystem Assessment 2005).
53 Whilst much attention has been devoted to separately studying the impacts of climate change
54 and biodiversity loss, considerably less is known about their interactive effects on ecosystems
55 and the services they provide (Balvanera, Pfisterer, Buchmann, He, Nakashizuka et al. 2006).
56 Studies which include the effects of climate change factors on the functioning of the
57 belowground compartment are even fewer and by and large highlight the considerable lack of
58 knowledge in this area (Chung, Zak, Reich & Ellsworth 2007, Kardol, Cregger, Campany &
59 Classen. 2010). Since the biological functioning and the species composition of the
60 belowground compartment is dependent on organic matter inputs from photosynthesising
61 plants, the understanding of the links between producer and decomposer subsystems is
62 essential for predicting of ecosystem-level responses to global change (Wardle 2002).

63 Grasslands constitute a widespread type of ecosystem covering ~ 27% of terrestrial
64 surface (Henwood 1998), highlighting their importance as model systems used for
65 investigating the consequences of biodiversity loss (Roscher, Temperton, Scherer-Lorezen,
66 Schmitz, Schumacher et al. 2005, Tilman, Reich & Knops 2006) or elevated CO₂ (Niklaus,
67 Alphei, Kampichler, Kandeler, Körner et al. 2007, Reich 2009). Reich, Knops, Tilman,
68 Craine, Ellsworth et al. (2001) showed that plant diversity interacts with elevated CO₂ and
69 nitrogen availability to modify ecosystem functioning. Furthermore, elevated atmospheric
70 CO₂ concentrations usually reduce stomatal density and conductance (Woodward & Bazzaz
71 1988; Tricker, Trewin, Kull, Clarkson, Eensalu et al. 2005) which may result in increased soil
72 moisture by reducing evapotranspiratory loss of water (Morgan et al. 2001; Nelson, Morgan,
73 Lecain, Mosier, Milchunas et al. 2004) under elevated levels of CO₂. Although both CO₂ and

plant diversity loss have been shown to impact the decomposer functioning through changes in the quality and quantity of organic matter inputs and impacts on soil moisture and nutrient availability, we have a limited understanding of their combined effects on the decomposer functioning (Niklaus et al. 2007) despite their important role in biogeochemical cycling, plant performance and soil C storage (Bardgett 2005).

Both earthworms and microorganisms are key decomposer groups sensitive to changes in the aboveground inputs, as well as soil water and nutrient regimes. Currently we have only a limited mechanistic understanding of the interactive effects of elevated CO₂ and plant diversity loss on their performance. The few studies which report effects of elevated CO₂ on earthworms predominantly measure their response in terms of activity, indicating increased activity with increasing levels of CO₂ (Yeates, Tate & Newton 1997, Arnone & Zaller 1997; Zaller & Arnone 1999c). However, an observation of community biomass and composition by Zaller & Arnone (1999c) found no effects of elevated CO₂. Available data also show that increasing plant species diversity (Zaller & Arnone 1999b; Speh, Josh, Schmid, Alphei & Körner 2000; Niklaus et al. 2007) has a positive effect on earthworm biomass and activity, but depends on species-specific relationships with earthworms (Zaller & Arnone 1999c; Milcu, Partsch, Langel & Scheu 2006). Milcu et al. (2006) suggested that changes in the quality rather than quantity of plant inputs associated with changes in species diversity affect earthworm performance. The consequences of elevated CO₂ on microorganisms are still unclear due to insufficient data to predict how microbial biomass and functioning change as atmospheric CO₂ concentration continues to rise (Zak et al 2000, Bardgett, Freeman & Ostle YEAR). The effect of diversity loss on soil microorganisms is somewhat clearer with recent publications pointing out the positive relationship between plant diversity and the functioning and biomass of microbial communities (Eisenhauer et al 2009).

The scope for interacting effects of diversity loss and elevated CO₂ on soil organisms is clearly large. Climatic change is likely to both directly and indirectly (via plant responses) affect soil biota, with associated consequences for ecosystem functioning. In this study we explored the interaction between plant species richness, elevated CO₂ and the presence of earthworms and its effects on the belowground compartment of model grassland, using a microcosm greenhouse experiment. Our overarching hypothesis is that the frequently observed beneficial effects of increasing the richness of plant species on earthworm performance and microbial biomass will be altered by elevated CO₂, through changes in belowground organic matter inputs and altered soil water and nitrogen availability.

Materials and methods

Experimental set up

Experimental grassland communities of varied plant species richness (1, 4 and 8 species) were established in cylindrical microcosms made of PVC tubes, 10.3 cm in diameter and 25 cm in height. The microcosms were sealed at the bottom with 0.5mm² mesh, and a layer of sand (0.25kg) was added to the bottom of the microcosms to ensure efficient drainage. The sand was topped by 1.6 kg of sieved soil (1cm diameter sieve) taken from the A horizon of a mesotrophic grassland (MG6 – *Lolium perenne* - *Cynosurus cristatus*; Rodwell 1998) in Silwood Park, Ascot, UK (N 51° 24.3 W 00° 38.5). Prior to use, the soil (bagshot sands, pH 5.35) was homogenized and defaunated by freezing at -22°C for two weeks (Huhta, Wright & Coleman 1989). Subsequently, the soil was watered intermittently over a 12 day period with deionised water to remove excess nutrients released by the perturbation.

The microcosms were planted out with eight seedlings (~ 5 cm height) pre-germinated in John Innes compost from a species pool of 37 grassland species (Appendix 1). The microcosms were assigned to eight air-conditioned chambers (100cm x 100cm x 53.5

cm) located in a greenhouse. An elevated atmospheric CO₂ treatment of 600 ppm simulating the average prediction for 2100 (IPCC 2007) was applied to four of the chambers, with the other four receiving air with ambient concentration of ~400 ppm.

The earthworm treatment (EW) was established by adding one *Lumbricus terrestris* L. (4.9774 ± 0.067g FW) to one set of the microcosms in each chamber. *L. terrestris* was chosen as a typical representative colonising the grassland type (MG6) from which the soil was extracted. As *L. terrestris* is an anecic litter feeding species, 3g DW of *Dactylis glomerata* litter (cut into 3cm pieces) was placed onto the soil surface in each microcosm. No additional litter entered the microcosms as any plant litter originating from the aboveground biomass was removed weekly. Each growth chamber thus contained 36 microcosms: two replicates of each species mixture, one containing a *L. terrestris* individual (EW) and one control (NW). The resulting total of 288 microcosms (3 plant diversity levels × 6 replicates/diversity level × 2 earthworm treatments × 2 CO₂ levels × 4 replicates per CO₂ level) were fitted with 15cm high cylindrical transparent plastic guards at the top of each microcosm to contain the earthworms in the microcosms. The greenhouse was lit naturally, but also received additional light for 8 hours a day via 400 Watt overhead 'POOT' lamps. Identical environmental conditions were maintained in all chambers, apart from the 4 chambers where atmospheric CO₂ was maintained at 600ppm for 8 hours during the photosynthetically active part of the day. Air humidity, air temperature and CO₂ levels of each chamber were monitored continuously. The microcosms were watered every 3 days, initially with 30ml of deionised water, increasing to 60ml, 80ml and then 120ml over the course of the experiment to account for increasing water use by growing plants. The positions of the microcosms within the growth chambers were randomised every 14 days.

Plant diversity experimental design

A pool of 37 South-East England mesotrophic acid grassland plant species belonging to three functional groups (grasses, herbs and legumes) was used to produce a total of 18 different species mixtures (see Appendix A) using independent random draws with replacement (i.e. species mixtures were created by randomly selecting species from the species pool with all species being available for to be drawn for any species mixture). The established gradient of species diversity (1, 4 and 8) had 6 replicates per diversity level. Any sampling effects were reduced by using species pool much larger than the maximum diversity level (Aarssen 1997).

Sampling and analytical procedures

The experiment has been harvested after four months of CO₂ exposure when the majority of plants showed signs of senescence. Immediately prior to destructive sampling, the soil moisture level of each microcosm was measured (ThetaProbe Soil Moisture Sensor - ML2x, Delta-T Products). Soil C/N content was analysed using a CNS elemental analyser (Thermo Scientific FlashEA 1112 series). Earthworms were hand collected during the final harvest, washed, dried for 1 min on filter paper and weighed. A sub-sample of soil was sieved to remove any roots (2mm dia.) and stored at 4°C. After 2 weeks the soil was used to assess the microbial biomass using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978) using electrolytic O₂ microcompensation apparatus (Scheu 1992). The microbial respiratory response to the addition of glucose was measured at 30-minute intervals for 24h at 22°C. Sufficient amount of glucose in solution was added to the substrate in order to saturate the catabolic activity of microorganisms (4 mg glucose per 1g of soil dry weight). The maximum initial respiratory response (MIRR [$\mu\text{g O}_2\cdot\text{h}^{-1}\cdot\text{g soil dw}^{-1}$]) was calculated as the average of the lowest three readings within the first 11 h and microbial biomass (C_{mic} [$\mu\text{g C}_{\text{mic}}\cdot\text{g soil dw}^{-1}$]) was calculated as $\text{MIRR} \times 38 \times 0.7$ (Anderson & Domsch 1978).

174 *Statistical analysis*

175 Mixed effects models, as implemented in the R Statistical package (lme function, R version
 176 2.10.1), was used to analyse the fixed effects of CO₂ (CO₂), earthworm presence (EW), plant
 177 species richness (SR) and their interactions on earthworm biomass, microbial biomass, soil
 178 moisture and soil N and C/N ratio. The random-effects of the maximal model, fit by
 179 maximum likelihood, had a microcosm within chamber random structure to account for the
 180 nested design [model <-lme (y ~ CO₂*EW*SR, random = ~1 | chamber / microcosmID),
 181 where y indicates the response variable, chamber represents the eight growth chambers at
 182 which the CO₂ treatment has been applied and microcosmID represents the individual
 183 microcosms]. Minimum adequate models were achieved by sequentially excluding non-
 184 significant terms (starting with highest-order interactions) and comparing each model with its
 185 predecessor using Akaike information criterion (Crawley 2007). Tukey's HSD posthoc test
 186 was used to perform pairwise comparison of treatments with more than two levels. Several
 187 continuous explanatory variables such as plant biomass (shoot, root, total), soil moisture and
 188 soil N content were tested as covariables in the models for identifying potential mechanisms.
 189 We used Statistica 8 package (StatSoft Inc) for graphical presentation of data.

190

191 **Results**

192 *Earthworm body weight.* In total, 81 % of the 144 *Lumbricus terrestris* used were retrieved at
 193 the end of the experiment and on average, their biomass decreased by 32%. Neither the CO₂
 194 nor plant species richness treatments had a significant effect on earthworm biomass (Table 1).
 195 They did, however, have a significant interactive effect (CO₂ x SR; Table 2) with earthworms
 196 exhibiting lower biomass in the eight species mixtures at ambient (-37.8%) than at elevated
 197 CO₂ (-29.5%) (Fig. 1a).

198 *Soil N.* Soil nitrogen content at the end of the experiment was not affected by the CO₂
 199 treatment alone or by the presence of earthworms (Table 1). Increasing plant species richness
 200 led to significantly lower soil N (Table 1), with the lowest N concentration in the eight
 201 species mixtures (0.13% N) compared to one and four species mixtures (0.14% N).

202 *Soil C/N ratio.* Increasing plant diversity led to significantly higher C/N ratio (Fig. 1b).
 203 Furthermore, the C/N ratio was affected by the interaction between CO₂ and earthworms
 204 (CO₂ x EW) with the lowest C/N ratio in the presence of legumes under ambient CO₂
 205 conditions.

206 *Soil moisture content.* Elevated CO₂ increased the soil moisture measured at the end of the
 207 experiment from 25.3% in the ambient CO₂ to 29.3%. Increasing plant species diversity led
 208 to significantly lower soil moisture in the four (26.1%) and eight (25.9%) species richness
 209 compared to monocultures (30.1%, Table 1). Soil moisture was also affected by the
 210 interaction between CO₂ concentration and the presence of earthworms (CO₂ x EW; Table 1
 211 and Figure 1c), with significantly less available soil water in the earthworm treatment and
 212 with the lowest amount of available soil water content at ambient CO₂ in the presence of
 213 earthworms (Tukey HSD <0.05).

214 *Microbial biomass.* Elevated CO₂ marginally increased microbial biomass (+6.2%) (Table 1).
 215 Plant species richness also affected the microbial biomass, with significantly higher biomass
 216 in the four species richness (226.0 mg Cmic g⁻¹ soil dry weight) compared to monocultures
 217 (208.2 mg Cmic g⁻¹ soil dry weight, Tukey HSD <0.05), with the eight species mixtures
 218 being intermediate (217.5 mg Cmic g⁻¹ soil dry weight, Tukey HSD >0.05). Additionally, the
 219 CO₂ treatment, earthworm presence and plant species richness had an interactive effect (CO₂
 220 x EW x SR; Table 2, Fig. 2) on the microbial biomass; under elevated CO₂ only four and
 221 eight species mixtures increased microbial biomass (Tukeys HSD <0.05) compared to

monocultures if earthworms were present, while in the absence of earthworms microbial biomass was higher in the monocultures (Fig. 2).

Discussion

Despite their small scale and somewhat artificial nature, microcosm experiments have a good track record of providing an indication of processes and mechanisms directing ecosystem functioning at much larger scales (Benton, Solan, Travis & Sait 2007). Given that all microcosms were constructed by using homogenised soil with standardised plant litter and no additional litter entered the soil, we contend that the observed effects of elevated CO₂ were occurring through the rhizosphere. Although the experiment discussed in this paper lasted only for the equivalent of one vegetative season, the observations show several interactive effects of elevated CO₂, earthworm presence and plant diversity on the functioning of the belowground compartment of a model temperate grassland.

Earthworms

It is widely accepted that the main drivers through which elevated CO₂ will affect soil organisms are soil moisture (Niklaus et al. 2007) and plant derived organic matter inputs such as litter and rhizodeposition (O'Neill 1994, Meehan et al. 2010). Changes in both quantity and quality of soil organic matter inputs have been shown to change with increasing plant diversity, due to alterations in the amount and chemical composition of organic matter entering the soil. This may potentially have beneficial effects on the earthworm community (Edwards 1996; Milcu, Partsch, Scherber, Weisser & Scheu 2008) though the relationship between plant species diversity and earthworm performance is unlikely to be straightforward with idiosyncratic effects often reported (Wardle, Bonner, Barker, Yeates, Nicholson et al. 1999; Eisenhower, Milcu, Sabais, Bessler, Weigelt et al. 2009). We observed higher

earthworm biomass in microcosms with higher plant diversity (8 species), but only under elevated CO₂ treatment. However, since soil moisture, plant shoot, root and total biomass, when used as potential covariables in the statistical model, did not render the CO₂ × SR interaction non-significant, we contend that the increase in earthworm biomass under elevated CO₂ is not a result of changes in soil moisture or plant biomass. As no aboveground inputs entered the system, the greater earthworm biomass could only have resulted from additional belowground inputs. Elevated CO₂ is known to generally enhance N rhizodeposition (Schultze & Merbach 2008) and N₂ fixation in legumes (Stephanie, Fischinger, Hristozkova, Mainassara & Schultze 2009) leading to increased total belowground N transport. This likely explains the higher biomass of *L. terrestris*, an anecic litter feeding species which has been previously shown to be closely linked to N availability (Milcu et al. 2008). Interestingly, the lowest biomass of earthworms was observed at the highest plant diversity level under ambient CO₂. This is in contradiction with existing literature which documents positive or idiosyncratic (Hedlund, Santa Regina, Van Der Putten, Lepš, Diaz et al. 2003; Eisenhauer et al. 2009), but no detrimental effects of increasing plant diversity on earthworm performance. The observed decrease in earthworm biomass was likely caused by the high competition for N between plants and earthworms as documented by the lower soil N and higher C/N ratio in the eight species diversity mixtures in an already low-N soil (0.13% N) (Fig. 1b). Indeed fitting the C/N ratio in the model renders the CO₂ × SR interaction non-significant ($P > 0.05$) and increases the fitness of the model as estimated by the AIC.

Microbial biomass

Like earthworms, soil microorganisms are often found to respond to elevated

CO₂ by changing their community composition, overall biomass and activity (De Graaff, Mavan Groenigen, Six, Hungate & Van Kessel 2006; Blagodatskaya, Blagodatsky, Dorodnikov & Kuzyakov 2010). The magnitude and the sign of the effect of elevated CO₂ on microorganisms is not very clear, with no effects (Holmes et al 2006), detrimental (Hungate et al. 1996) or idiosyncratic responses having been documented (Zak, Pregitzer, King & Holmes 2000; Kanerva, Palojarvi, Rämö & Manninen. 2008). Here we only found a marginally increased microbial carbon biomass (C_{mic}) in the elevated CO₂. Changes in litter inputs, root biomass and production, soil moisture and rhizodeposition have been previously identified to affect microbial biomass under elevated CO₂ (Zak 2000). However, whilst we could not identify the exact mechanism, it is worth noting that this increase was mediated by belowground effects of elevated CO₂ as no aboveground plant litter entered the soil. Moreover, we found significant changes in response to plant diversity. Higher C_{mic} at higher plant diversity levels have recently been reported in a 7 year experiment (Eisenhauer et al. 2009). In our microcosms, we found the eight and four plant species mixtures to have higher C_{mic} than the monocultures. However, contrary to our expectations, the highest biomass was found in the four and not in the eight plant species mixtures. Higher C_{mic} at intermediate plant diversity levels have been previously found in early stages of experiments manipulating diversity as the effects of plants on the C_{mic} increased with time (Eisenhauer et al. 2009).

The presence of animal ecosystem engineers such as anecic earthworms also affects the functioning of the belowground systems and microbial biomass has been noted to increase due to accelerated incorporation of nutrient rich litter into the soil. Sheehan, Kirwan, Connolly & Bolger (2008) suggested that the positive effect of anecic species on microbial biomass was greatest with increased food supply. We found that microbial biomass varied with the presence of earthworms, plant species richness and CO₂ level; higher microbial biomass was found under elevated CO₂ at higher plant diversity levels when earthworms

were present, but also at the lower diversity level in the absence of earthworms. Given the inconclusiveness in observed effects, we could not identify the exact mechanism behind this interaction as none of the measured covariates (root biomass, total plant biomass, soil moisture or soil N content) affected the significance level of the interaction. This highlights the complexity of belowground C fluxes, it is possible that a longer-term experiment might be able to uncover such a mechanism by allowing the systems to stabilise and transient effects to lose their influence.

Soil moisture

Water availability frequently limits the functioning of the detritivore system (Swift, Heal & Anderson 1979; Austin 2002) with many soil decomposers migrating into deeper soil layers or to entering a dormant stage under water stress, with consequences for the functioning of the ecosystems. Water availability is essential for normal earthworm physiology due to their cutaneous mode of respiration and for maintaining their coelomic hydrostatic pressure at levels that allow locomotion (Lavelle 1988). Although highly dependent on soil water, earthworm activities (e.g. burrowing, casting, midden formation) can affect the soil water dynamics in a feedback loop through effects on soil physical properties. The mechanisms include changes in soil porosity and stability of organomineral aggregates. By burrowing and by the creation of organomineral casts, earthworms are generally thought to exert a beneficial influence on soil water availability through increased water infiltration and by improving soil water holding capacity (Edwards 2004). However, negative effects of earthworm activity on soil water retention have also been reported (Blouin, Lavelle & Laffray 2007) and there is increasing evidence that elevated CO₂ affects belowground communities also through improved plant and soil water status due to reduced evapotranspiration via reduced leaf conductance (Niklas et al 2007). This could play an important role in the functioning of the

ecosystems particularly in conditions of water shortage when burrows increases water drainage and evaporative water loss. Our results are in line with the findings of Blouin et al. (2007), showing a significant decrease in soil moisture content in the presence of *L. terrestris* via preferential water flow through the vertical burrows which can account to up to 10% of the water flow (Edwards, Shipitalo, Owens & Norton 1989). Given that in our experiment the soil water content was reduced by earthworms in both CO₂ treatments, but that a smaller reduction was observed under to elevated CO₂ (Fig. 1c), this suggests that both reduced soil water retention and increased water use efficiency (Wullschleger, Tschaplinski & Norby 2002) contribute to the observed effects. However, it might not be possible to extrapolate our this finding to field conditions due to differences in drainage and water movement between microcosms and undisturbed soil profile. Our observations indicate that deep vertical macropores created by anecic earthworms may modify the effect of elevated CO₂ on soil water availability, the mechanism behind this interaction remains to be verified in the field.

Soil nitrogen

In an extensive meta-analysis considering the effects of elevated CO₂ on nutrient cycling De Graaff et al. (2006) found no effects on N mineralisation rates, but documented increased soil C inputs leading to increased soil C/N ratio. Although we found no significant effect of CO₂ on the soil N or C/N ratio, we observed a decrease of soil N and an increase in the soil C/N ratio with increasing plant diversity. These kind of effects have previously been reported (e.g. Oelmann, Wilcke, Temperton, Buchmann, Roscher et al 2007 and Roscher, Thein, Schmid & Scherer Lorenzen 2008) and they are attributed to a better usage of the total resources, whether they be nitrogen or water (i.e. increased complementarity). This mechanism contributes to the positive relationship between species richness and above-ground biomass production and is often paralleled by a decrease in

belowground available N due to increased efficiency in exploiting the available resource pool but facilitating an increased accumulation of plant biomass as compared to low-diversity communities (Roscher et al 2008). In support of this we found an increase of total plant biomass with increasing diversity ($F_{2,270} = 6.6294$, $p = 0.002$).

Earthworm presence is known to increase N cycling and availability (Scheu 2003; Edwards 2004), however, in this study the presence of earthworms did not have any effect on the availability of soil N. In our experiment this is presumably related to the low resource (litter) inputs in our system, but we acknowledge that by measuring only changes in total soil N we could have missed potential changes in available N.

In conclusion, the findings of this experiment emphasize several interacting mechanisms by which elevated CO₂ can modify the established relationship between plant diversity, earthworms and microorganisms. The results confirm our over-arching hypothesis that changes in belowground resource allocation, soil water and N availability mediated by elevated CO₂ alter the relationship between plant diversity and the functioning of the belowground compartment. Some of the observed interactions might be transient or magnified by the nature of this microcosm experiment. However, these results confirm the existence of several mechanisms which could determine the response and adaptation of essential soil functions such as decomposition, nutrient cycling, carbon storage, water filtration and primary productivity to elevated CO₂ at the ecosystem level. Such mechanisms and interactions need further investigation in order to understand and predict the interactive effects of climate change and diversity loss on ecosystem functioning and stability.

Acknowledgements

We thank NERC for funding the placement student. We gratefully acknowledge Prof. Stefan Scheu for allowing performing the microbial biomass analyses in his lab. We also thank Prof. Mick Crawley for comments on the R statistical analysis, Dennis Wildman and Tom Sloan for their invaluable technical support and Nico Eisenhauer and Alessandra Lagomarsino for their comments on the manuscript.

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Table 1. Output table of mix-effects models for the effect of CO₂ treatment (CO₂), earthworm presence (EW), plant species richness (SR) and their interactions on the belowground response variables. Excluded effects refer to the treatment factors which have been removed from the model in order to achieve minimum adequate models (Crawley 2007).

Belowground measurements															
	Earthworm biomass			Soil moisture			Soil N			Soil C to N ratio			Microbial biomass		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
CO ₂	1,6	0.32	0.591	1,6	11.12	0.015	1,6	0.1	0.947	1,6	0.48	0.514	1,6	4.69	0.073
EW	n.a	n.a	n.a	1,273	108.7	<.001	1,253	1.1	0.284	1,256	2.75	0.098	1,256	0.9	0.342
SR	2,101	1.61	0.205	2,273	7.9	<.001	2,253	0.161	0.021	2,256	3.54	0.030	2,256	3.1	0.047
CO ₂ × EW	excl.	excl.	excl.	1,273	9.7	0.002	1,253	0.1	0.777	excl.	excl.	excl.	1,256	0.1	0.969
CO ₂ × SR	2,101	3.54	0.032	excl.	excl.	excl.	excl.	excl.	excl.	2,256	1.59	0.206	2,256	1.77	0.172
CO ₂ × EW × SR	excl.	excl.	excl.	excl.	excl.	excl.	excl.	excl.	excl.	excl.	excl.	excl.	2,256	4.0	0.018

Figure legends

Figure 1. Two-way interaction effects of plant species richness (SR) and atmospheric CO₂ treatment on earthworm mass loss (A), effects of SR on soil C/N ratio (B) and two-way interaction effects of SR and earthworm presence on soil moisture (C). Error bars represent $\pm$ SE.

Figure 2. Three-way interaction effects between plant species richness (SR), atmospheric CO₂ and earthworm presence on the carbon (glucose) induced microbial biomass (Cmic). Error bars represent $\pm$ SE

Figure 1

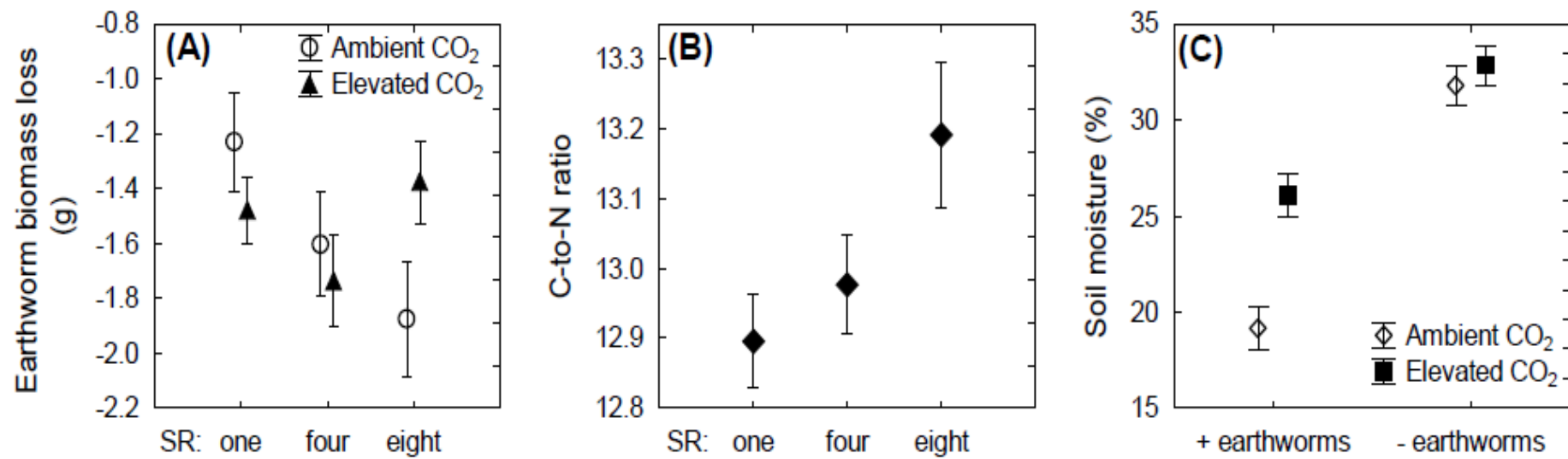
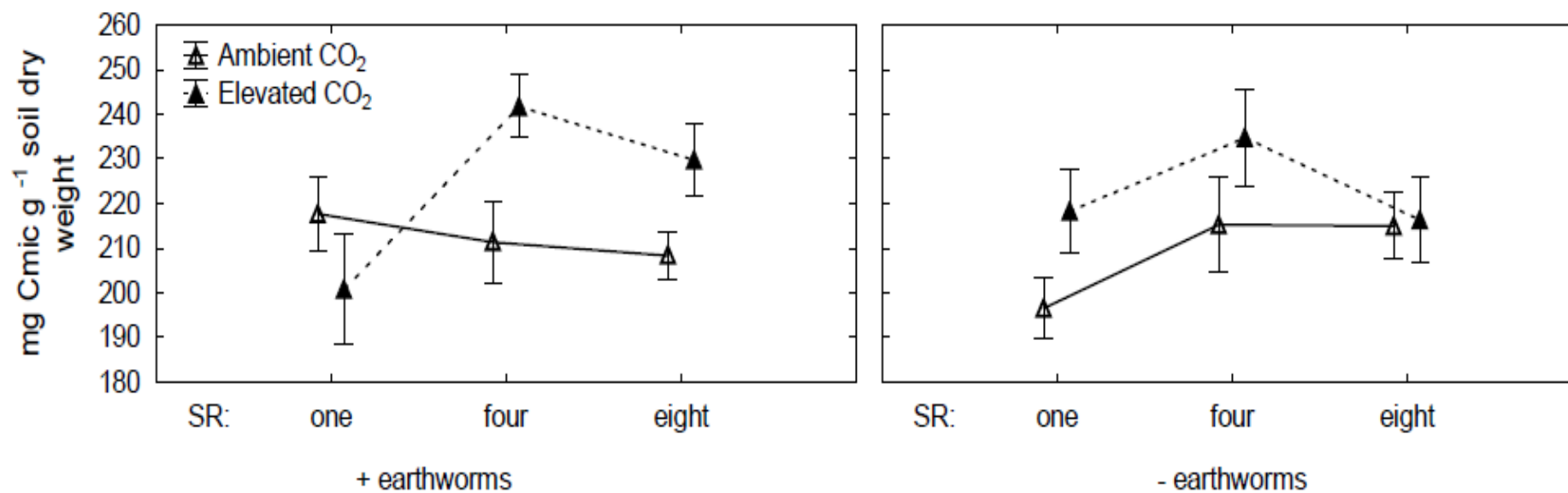


Figure 2



Appendix A. Experimental design layout of the 18 different species mixtures. Grasses are shown in yellow, herbs in green and legumes in dark green.

Nr	Diversity level	Plant Species in Microcosm							
1	Monoculture	<i>Bromus hordeaceus</i>	Grass Herb Legume						
2	Monoculture	<i>Cynosurus cristatus</i>							
3	Monoculture	<i>Achillea millefolium</i>							
4	Monoculture	<i>Dipsacus fullonum</i>							
5	Monoculture	<i>Trifolium repens</i>							
6	Monoculture	<i>Vicia cracca</i>							
7	4-species-mixture	<i>Holcus lanatus</i>	<i>Lolium perenne</i>	<i>Vulpia bromoides</i>	<i>Festuca pratensis</i>				
8	4-species-mixture	<i>Taraxacum officinale</i>	<i>Rumex acetosella</i>	<i>Dipsacus fullonum</i>	<i>Daucus carota</i>				
9	4-species-mixture	<i>Trifolium dubium</i>	<i>Trifolium pratense</i>	<i>Medicago lupulina</i>	<i>Vicia sativa</i>				
10	4-species-mixture	<i>Bromus hordeaceus</i>	<i>Lolium perene</i>	<i>Leontodon hispidus</i>	<i>Vicia sativa</i>				
11	4-species-mixture	<i>Bromus sterilis</i>	<i>Plantago lanceolata</i>	<i>Taraxacum officinale</i>	<i>Lotus corniculatus</i>				
12	4-species-mixture	<i>Alopecurus pratensis</i>	<i>Myosotis arvensis</i>	<i>Trifolium pratense</i>	<i>Lathyrus tuberosus</i>				
13	8-species-mixture	<i>Holcus lanatus</i>	<i>Anthoxanthu m odoratum</i>	<i>Festuca pratensis</i>	<i>Alopecurus pratensis</i>	<i>Agrostis capillaris</i>	<i>Festuca rubra</i>	<i>Bromus sterilis</i>	<i>Poa annua</i>
14	8-species-mixture	<i>Galium mollugo</i>	<i>Plantago lanceolata</i>	<i>Matricaria recutita</i>	<i>Daucus carota</i>	<i>Prunella vulgaris</i>	<i>Leontodon hispidus</i>	<i>Myosotis arvensis</i>	<i>Rumex acetosella</i>
15	8-species-mixture	<i>Trifolium dubium</i>	<i>Vicia sativa</i>	<i>Lathyrus pratensis</i>	<i>Vicia cracca</i>	<i>Lotus corniculatus</i>	<i>Medicago sativa</i>	<i>Trifolium medium</i>	<i>Medicago lupulina</i>
16	8-species-mixture	<i>Elytrigia repens</i>	<i>Anthoxanthu m odoratum</i>	<i>Festuca rubra</i>	<i>Plantago lanceolata</i>	<i>Prunella vulgaris</i>	<i>Dipsacus fullonum</i>	<i>Medicago lupulina</i>	<i>Lotus corniculatus</i>
17	8-species-mixture	<i>Lolium perene</i>	<i>Bromus sterilis</i>	<i>Achillea millefolium</i>	<i>Matricaria recutita</i>	<i>Leucanthem um vulgare</i>	<i>Vicia sativa</i>	<i>Trifolium medium</i>	<i>Medicago sativa</i>
18	8-species-mixture	<i>Dactylis glomerata</i>	<i>Holcus lanatus</i>	<i>Festuca pratensis</i>	<i>Leucanthem um vulgare</i>	<i>Achillea millefolium</i>	<i>Trifolium dubium</i>	<i>Vicia cracca</i>	<i>Lathyrus tuberosus</i>