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8

9   Belowground interactive effects of elevated CO_2, plant diversity and

10   earthworms in model grasslands

11

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ABSTRACT

The potential interactive effects of future atmospheric CO_2 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 CO_2 (ambient and + 200 p.p.m.v. CO_2) 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 CO_2 through impacts on belowground organic matter inputs, soil water availability and nitrogen availability. We found higher earthworm biomass in eight species mixtures under elevated CO_2, and higher microbial biomass under elevated CO_2 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 CO_2 and plant diversity on the belowground compartment. The interacting mechanisms by which elevated CO_2 modified the impact of plant diversity on earthworms and microorganisms are discussed.

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-Lorenzen,

66 Schmitz, Schumacher et al. 2005, Tilman, Reich & Knops 2006) or elevated CO_2 (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 CO_2 and

69 nitrogen availability to modify ecosystem functioning. Furthermore, elevated atmospheric

70 CO_2 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 CO_2. Although both CO_2 and

74   plant diversity loss have been shown to impact the decomposer functioning through changes

75   in the quality and quantity of organic matter inputs and impacts on soil moisture and nutrient

76   availability, we have a limited understanding of their combined effects on the decomposer

77   functioning (Niklaus et al. 2007) despite their important role in biogeochemical cycling, plant

78   performance and soil C storage (Bardgett 2005).

79   Both earthworms and microorganisms are key decomposer groups sensitive to

80   changes in the aboveground inputs, as well as soil water and nutrient regimes. Currently we

81   have only a limited mechanistic understanding of the interactive effects of elevated CO_2 and

82   plant diversity loss on their performance. The few studies which report effects of elevated

83   CO_2 on earthworms predominantly measure their response in terms of activity, indicating

84   increased activity with increasing levels of CO_2 (Yeates, Tate & Newton 1997, Arnone &

85   Zaller 1997; Zaller & Arnone 1999c). However, an observation of community biomass and

86   composition by Zaller & Arnone (1999c) found no effects of elevated CO_2. Available data

87   also show that increasing plant species diversity (Zaller & Arnone 1999b; Speh, Josh,

88   Schmid, Alphei & Körner 2000; Niklaus et al. 2007) has a positive effect on earthworm

89   biomass and activity, but depends on species-specific relationships with earthworms (Zaller

90   & Arnone 1999c; Milcu, Partsch, Langel & Scheu 2006). Milcu et al. (2006) suggested that

91   changes in the quality rather than quantity of plant inputs associated with changes in species

92   diversity affect earthworm performance. The consequences of elevated CO_2 on

93   microorganisms are still unclear due to insufficient data to predict how microbial biomass

94   and functioning change as atmospheric CO_2 concentration continues to rise (Zak et al 2000,

95   Bardgett, Freeman & Ostle YEAR). The effect of diversity loss on soil microorganisms is

96   somewhat clearer with recent publications pointing out the positive relationship between

97   plant diversity and the functioning and biomass of microbial communities (Eisenhauer et al

98   2009).

99   The scope for interacting effects of diversity loss and elevated CO_2 on soil organisms

100   is clearly large. Climatic change is likely to both directly and indirectly (via plant responses)

101   affect soil biota, with associated consequences for ecosystem functioning. In this study we

102   explored the interaction between plant species richness, elevated CO_2 and the presence of

103   earthworms and its effects on the belowground compartment of model grassland, using a

104   microcosm greenhouse experiment. Our overarching hypothesis is that the frequently

105   observed beneficial effects of increasing the richness of plant species on earthworm

106   performance and microbial biomass will be altered by elevated CO_2, through changes in

107   belowground organic matter inputs and altered soil water and nitrogen availability.

108

Materials and methods

110   *Experimental set up*

111   Experimental grassland communities of varied plant species richness (1, 4 and 8 species)

112   were established in cylindrical microcosms made of PVC tubes, 10.3 cm in diameter and 25

113   cm in height. The microcosms were sealed at the bottom with $0.5mm^2$ mesh, and a layer of

114   sand (0.25kg) was added to the bottom of the microcosms to ensure efficient drainage. The

115   sand was topped by 1.6 kg of sieved soil (1cm diameter sieve) taken from the A horizon of a

116   mesotrophic grassland (MG6 − Lolium perene - Cynosurus cristatus; Rodwell 1998) in

117   Silwood Park, Ascot, UK (N 51° 24.3 W 00° 38.5). Prior to use, the soil (bagshot sands, pH

118   5.35) was homogenized and defaunated by freezing at $-22^{o}C$ for two weeks (Huhta, Wright &

119   Coleman 1989). Subsequently, the soil was watered intermittently over a 12 day period with

120   deionised water to remove excess nutrients released by the perturbation.

121   The microcosms were planted out with eight seedlings (~ 5 cm height) pre-

122   germinated in John Innes compost from a species pool of 37 grassland species (Appendix 1).

123   The microcosms were assigned to eight air-conditioned chambers (100cm x 100cm x 53.5

124   cm) located in a greenhouse. An elevated atmospheric CO_2 treatment of 600 ppm simulating

125   the average prediction for 2100 (IPCC 2007) was applied to four of the chambers, with the

126   other four receiving air with ambient concentration of ~400 ppm.

127   The earthworm treatment (EW) was established by adding one *Lumbricus terrestris* L.

128   (4.9774 ± 0.067g FW) to one set of the microcosms in each chamber. *L. terrestris* was chosen

129   as a typical representative colonising the grassland type (MG6) from which the soil was

130   extracted. As *L. terrestris* is an anecic litter feeding species, 3g DW of *Dactylis glomerata*

131   litter (cut into 3cm pieces) was placed onto the soil surface in each microcosm. No additional

132   litter entered the microcosms as any plant litter originating from the aboveground biomass

133   was removed weekly. Each growth chamber thus contained 36 microcosms: two replicates of

134   each species mixture, one containing a *L. terrestris* individual (EW) and one control (NW).

135   The resulting total of 288 microcosms (3 plant diversity levels × 6 replicates/diversity level ×

136   2 earthworm treatments × 2 CO_2 levels × 4 replicates per CO_2 level) were fitted with 15cm

137   high cylindrical transparent plastic guards at the top of each microcosm to contain the

138   earthworms in the microcosms. The greenhouse was lit naturally, but also received additional

139   light for 8 hours a day via 400 Watt overhead 'POOT' lamps. Identical environmental

140   conditions were maintained in all chambers, apart from the 4 chambers where atmospheric

141   CO_2 was maintained at 600ppm for 8 hours during the photosynthetically active part of the

142   day. Air humidity, air temperature and CO_2 levels of each chamber were monitored

143   continuously. The microcosms were watered every 3 days, initially with 30ml of deionised

144   water, increasing to 60ml, 80ml and then 120ml over the course of the experiment to account

145   for increasing water use by growing plants. The positions of the microcosms within the

146   growth chambers were randomised every 14 days.

147

148   *Plant diversity experimental design*

149   A pool of 37 South-East England mesotrophic acid grassland plant species belonging to three

150   functional groups (grasses, herbs and legumes) was used to produce a total of 18 different

151   species mixtures (see Appendix A) using independent random draws with replacement (i.e.

152   species mixtures were created by randomly selecting species from the species pool with all

153   species being available for to be drawn for any species mixture). The established gradient of

154   species diversity (1, 4 and 8) had 6 replicates per diversity level. Any sampling effects were

155   reduced by using species pool much larger than the maximum diversity level (Aarssen 1997).

156

157   *Sampling and analytical procedures*

158   The experiment has been harvested after four months of CO_2 exposure when the majority of

159   plants showed signs of senescence. Immediately prior to destructive sampling, the soil

160   moisture level of each microcosm was measured (ThetaProbe Soil Moisture Sensor - ML2x,

161   Delta-T Products). Soil C/N content was analysed using a CNS elemental analyser (Thermo

162   Scientific FlashEA 1112 series). Earthworms were hand collected during the final harvest,

163   washed, dried for 1 min on filter paper and weighed. A sub-sample of soil was sieved to

164   remove any roots (2mm dia.) and stored at $4^{o}C$. After 2 weeks the soil was used to assess the

165   microbial biomass using the substrate induced respiration (SIR) method (Anderson and

166   Domsch, 1978) using electrolytic O_2 microcompensation apparatus (Scheu 1992). The

167   microbial respiratory response to the addition of glucose was measured at 30-minute intervals

168   for 24h at $22^{o}C$. Sufficient amount of glucose in solution was added to the substrate in order

169   to saturate the catabolic activity of microorganisms (4 mg glucose per 1g of soil dry weight).

170   The maximum initial respiratory response (MIRR [µg O2*h-1*g soil dw-1]) was calculated

171   as the average of the lowest three readings within the first 11 h and microbial biomass (C_{mic}

172   [µg C_{mic}*g soil dw-1]) was calculated as MIRR*38*0.7 (Anderson & Domsch 1978).

173

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 CO_2 (CO_2), 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 ~ CO_2*EW*SR, random = ~1 | chamber / microcosmID),

181   where y indicates the response variable, chamber represents the eight growth chambers at

182   which the CO_2 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 CO_2

194   nor plant species richness treatments had a significant effect on earthworm biomass (Table 1).

195   They did, however, have a significant interactive effect (CO_2 x SR; Table 2) with earthworms

196   exhibiting lower biomass in the eight species mixtures at ambient (-37.8%) than at elevated

197   CO_2 (-29.5%) (Fig. 1a).

198   *Soil N.* Soil nitrogen content at the end of the experiment was not affected by the CO_2

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 CO_2 and earthworms

204   (CO_2 x EW) with the lowest C/N ratio in the presence of legumes under ambient CO_2

205   conditions.

206   *Soil moisture content.* Elevated CO_2 increased the soil moisture measured at the end of the

207   experiment from 25.3% in the ambient CO_2 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 CO_2 concentration and the presence of earthworms (CO_2 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 CO_2 in the presence of

213   earthworms (Tukey HSD <0.05).

214   *Microbial biomass.* Elevated CO_2 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   CO_2 treatment, earthworm presence and plant species richness had an interactive effect (CO_2

220    $\times$ EW $\times$ SR; Table 2, Fig. 2) on the microbial biomass; under elevated CO_2 only four and

221   eight species mixtures increased microbial biomass (Tukeys HSD <0.05) compared to

222   monocultures if earthworms were present, while in the absence of earthworms microbial

223   biomass was higher in the monocultures (Fig. 2).

224

Discussion

226   Despite their small scale and somewhat artificial nature, microcosm experiments have a good

227   track record of providing an indication of processes and mechanisms directing ecosystem

228   functioning at much larger scales (Benton, Solan, Travis & Sait 2007). Given that all

229   microcosms were constructed by using homogenised soil with standardised plant litter and no

230   additional litter entered the soil, we contend that the observed effects of elevated CO_2 were

231   occurring through the rhizosphere. Although the experiment discussed in this paper lasted

232   only for the equivalent of one vegetative season, the observations show several interactive

233   effects of elevated CO_2, earthworm presence and plant diversity on the functioning of the

234   belowground compartment of a model temperate grassland.

235

236   *Earthworms*

237   It is widely accepted that the main drivers through which elevated CO_2 will affect soil

238   organisms are soil moisture (Niklaus et al. 2007) and plant derived organic matter inputs such

239   as litter and rhizodeposition (O'Neill 1994, Meehan et al. 2010). Changes in both quantity

240   and quality of soil organic matter inputs have been shown to change with increasing plant

241   diversity, due to alterations in the amount and chemical composition of organic matter

242   entering the soil. This may potentially have beneficial effects on the earthworm community

243   (Edwards 1996; Milcu, Partsch, Scherber, Weisser & Scheu 2008) though the relationship

244   between plant species diversity and earthworm performance is unlikely to be straightforward

245   with idiosyncratic effects often reported (Wardle, Bonner, Barker, Yeates, Nicholson et al.

246   1999; Eisenhower, Milcu, Sabais, Bessler, Weigelt et al. 2009). We observed higher

247   earthworm biomass in microcosms with higher plant diversity (8 species), but only under

248   elevated CO_2 treatment. However, since soil moisture, plant shoot, root and total biomass,

249   when used as potential covariables in the statistical model, did not render the $CO_2 \times SR$

250   interaction non-significant¸ we contend that the increase in earthworm biomass under

251   elevated CO_2 is not a result of changes in soil moisture or plant biomass. As no aboveground

252   inputs entered the system, the greater earthworm biomass could only have resulted from

253   additional belowground inputs. Elevated CO_2 is known to generally enhance N

254   rhizodeposition (Schultze & Merbach 2008) and N_2 fixation in legumes (Stephanie,

255   Fischinger, Hristozkova, Mainassara & Schultze 2009) leading to increased total

256   belowground N transport. This likely explains the higher biomass of *L. terrestris*, an anecic

257   litter feeding species which has been previously shown to be closely linked to N availability

258   (Milcu et al. 2008). Interestingly, the lowest biomass of earthworms was observed at the

259   highest plant diversity level under ambient CO_2. This is in contradiction with existing

260   literature which documents positive or idiosyncratic (Hedlund, Santa Regina , Van Der

261   Putten, Lepš, Diaz et al. 2003; Eisenhauer et al. 2009), but no detrimental effects of

262   increasing plant diversity on earthworm performance. The observed decrease in earthworm

263   biomass was likely caused by the high competition for N between plants and earthworms as

264   documented by the lower soil N and higher C/N ratio in the eight species diversity mixtures

265   in an already low-N soil (0.13% N) (Fig. 1b). Indeed fitting the C/N ratio in the model

266   renders the $CO_2 \times SR$ interaction non-significant ($P>0.05$) and increases the fitness of the

267   model as estimated by the AIC.

268

269   *Microbial biomass*

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

271   CO_2 by changing their community composition, overall biomass and activity (De Graaff,

272   Mavan Groenigen, Six, Hungate & Van Kessel 2006; Blagodatskaya, Blagodatsky,

273   Dorodnikov & Kuzyakov 2010). The magnitude and the sign of the effect of elevated CO_2 on

274   microorganisms is not very clear, with no effects (Holmes et al 2006), detrimental (Hungate

275   et al. 1996) or idiosyncratic responses having been documented (Zak, Pregitzer, King &

276   Holmes 2000; Kanerva, Palojärvi, Rämö & Manninen. 2008). Here we only found a

277   marginally increased microbial carbon biomass (Cmic) in the elevated CO_2. Changes in litter

278   inputs, root biomass and production, soil moisture and rhizodeposition have been previously

279   identified to affect microbial biomass under elevated CO_2 (Zak 2000). However, whilst we

280   could not identify the exact mechanism, it is worth noting that this increase was mediated by

281   belowground effects of elevated CO_2 as no aboveground plant litter entered the soil.

282   Moreover, we found significant changes in response to plant diversity. Higher Cmic at higher

283   plant diversity levels have recently been reported in a 7 year experiment (Eisenhauer et al.

284   2009). In our microcosms, we found the eight and four plant species mixtures to have higher

285   C*mic* than the monocultures. However, contrary to our expectations, the highest biomass was

286   found in the four and not in the eight plant species mixtures. Higher C_{mic} at intermediate plant

287   diversity levels have been previously found in early stages of experiments manipulating

288   diversity as the effects of plants on the C_{mic} increased with time (Eisenhauer et al. 2009).

289   The presence of animal ecosystem engineers such as anecic earthworms also affects

290   the functioning of the belowground systems and microbial biomass has been noted to

291   increase due to accelerated incorporation of nutrient rich litter into the soil. Sheehan, Kirwan,

292   Connolly & Bolger (2008) suggested that the positive effect of anecic species on microbial

293   biomass was greatest with increased food supply. We found that microbial biomass varied

294   with the presence of earthworms, plant species richness and CO_2 level; higher microbial

295   biomass was found under elevated CO_2 at higher plant diversity levels when earthworms

296   were present, but also at the lower diversity level in the absence of earthworms. Given the

297   inconclusiveness in observed effects, we could not identify the exact mechanism behind this

298   interaction as none of the measured covariates (root biomass, total plant biomass, soil

299   moisture or soil N content) affected the significance level of the interaction. This highlights

300   the complexity of belowground C fluxes, it is possible that a longer-term experiment might

301   be able to uncover such a mechanism by allowing the systems to stabilise and transient

302   effects to lose their influence.

303

304   *Soil moisture*

305   Water availability frequently limits the functioning of the detritivore system (Swift, Heal &

306   Anderson 1979; Austin 2002) with many soil decomposers migrating into deeper soil layers

307   or to entering a dormant stage under water stress, with consequences for the functioning of

308   the ecosystems. Water availability is essential for normal earthworm physiology due to their

309   cutaneous mode of respiration and for maintaining their coelomic hydrostatic pressure at

310   levels that allow locomotion (Lavelle 1988). Although highly dependent on soil water,

311   earthworm activities (e.g. burrowing, casting, midden formation) can affect the soil water

312   dynamics in a feedback loop through effects on soil physical properties. The mechanisms

313   include changes in soil porosity and stability of organomineral aggregates. By burrowing and

314   by the creation of organomineral casts, earthworms are generally thought to exert a beneficial

315   influence on soil water availability through increased water infiltration and by improving soil

316   water holding capacity (Edwards 2004). However, negative effects of earthworm activity on

317   soil water retention have also been reported (Blouin, Lavelle & Laffray 2007) and there is

318   increasing evidence that elevated CO_2 affects belowground communities also through

319   improved plant and soil water status due to reduced evapotranspiration via reduced leaf

320   conductance (Niklas et al 2007). This could play an important role in the functioning of the

321   ecosystems particularly in conditions of water shortage when burrows increases water

322   drainage and evaporative water loss. Our results are in line with the findings of Blouin et al.

323   (2007), showing a significant decrease in soil moisture content in the presence of *L. terrestris*

324   via preferential water flow through the vertical burrows which can account to up to 10% of

325   the water flow (Edwards, Shipitalo, Owens & Norton 1989). Given that in our experiment the

326   soil water content was reduced by earthworms in both CO_2 treatments, but that a smaller

327   reduction was observed under to elevated CO_2 (Fig. 1c), this suggests that both reduced soil

328   water retention and increased water use efficiency (Wullschleger, Tschaplinski & Norby

329   2002) contribute to the observed effects. However, it might not be possible to extrapolate our

330   this finding to filed conditions due to differences in drainage and mater movement between

331   microcosms and undisturbed soil profile. Our observations indicate that deep vertical

332   macropores created by anecic eartworms may modify the effect of elevated CO2 on soil

333   water availability, the mechanism behind this interaction remains to be verified in the field.

334

335   *Soil nitrogen*

336   In an extensive meta-analysis considering the effects of elevated CO_2 on nutrient

337   cycling De Graaff et al. (2006) found no effects on N mineralisation rates, but

338   documented increased soil C inputs leading to increased soil C/N ratio. Although

339   we found no significant effect of CO_2 on the soil N or C/N ratio, we observed a decrease of

340   soil N and an increase in the soil C/N ratio with increasing plant diversity. These kind of

341   effects have previously been reported (e.g. Oelmann,Wilcke, Temperton, Buchmann, Roscher

342   et al 2007 and Roscher, Thein, Schmid & Scherer Lorenzen 2008) and they are attributed to a

343   better usage of the total resources, whether they be nitrogen or water (i.e. increased

344   complementarity). This mechanism contributes to the positive relationship between species

345   richness and above-ground biomass production and is often paralleled by a decrease in

346 belowground available N due to increased efficiency in exploiting the available resource pool

347 but facilitating an increased accumulation of plant biomass as compared to low-diversity

348 communities (Roscher et al 2008). In support of this we found an increase of total plant

349 biomass with increasing diversity ($F_{2,270} = 6.6294$, p = 0.002).

350   Earthworm presence is known to increase N cycling and availability (Scheu 2003;

351 Edwards 2004), however, in this study the presence of earthworms did not have any effect on

352 the availability of soil N. In our experiment this is presumably related to the low resource

353 (litter) inputs in our system, but we acknowledge that by measuring only changes in total soil

354 N we could have missed potential changes in available N.

355

356   In conclusion, the findings of this experiment emphasize several interacting

357 mechanisms by which elevated CO_2 can modify the established relationship between plant

358 diversity, earthworms and microorganisms. The results confirm our over-arching hypothesis

359 that changes in belowground resource allocation, soil water and N availability mediated by

360 elevated CO_2 alter the relationship between plant diversity and the functioning of the

361 belowground compartment. Some of the observed interactions might be transient or

362 magnified by the nature of this microcosm experiment. However, these results confirm the

363 existence of several mechanisms which could determine the response and adaptation of

364 essential soil functions such as decomposition, nutrient cycling, carbon storage, water

365 filtration and primary productivity to elevated CO_2 at the ecosystem level. Such mechanisms

366 and interactions need further investigation in order to understand and predict the interactive

367 effects of climate change and diversity loss on ecosystem functioning and stability.

368

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Table 1. Output table of mix-effects models for the effect of CO_2 treatment (CO_2), 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	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
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**
$CO_2 \times$ 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
$CO_2 \times$ 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
$CO_2 \times$ EW $\times$ 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 CO_2 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 ± SE.

Figure 2. Three-way interaction effects between plant species richness (SR), atmopsperic CO_2 and earthworm presence on the carbon (glucose) induced microbial biomass (Cmic). Error bars represent ± SE
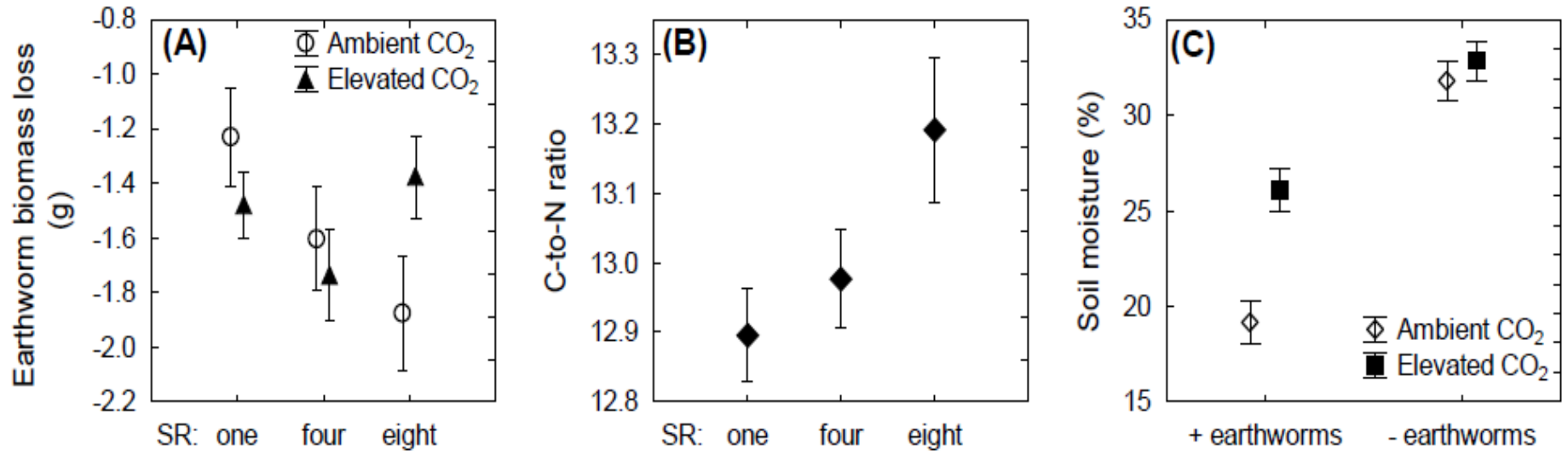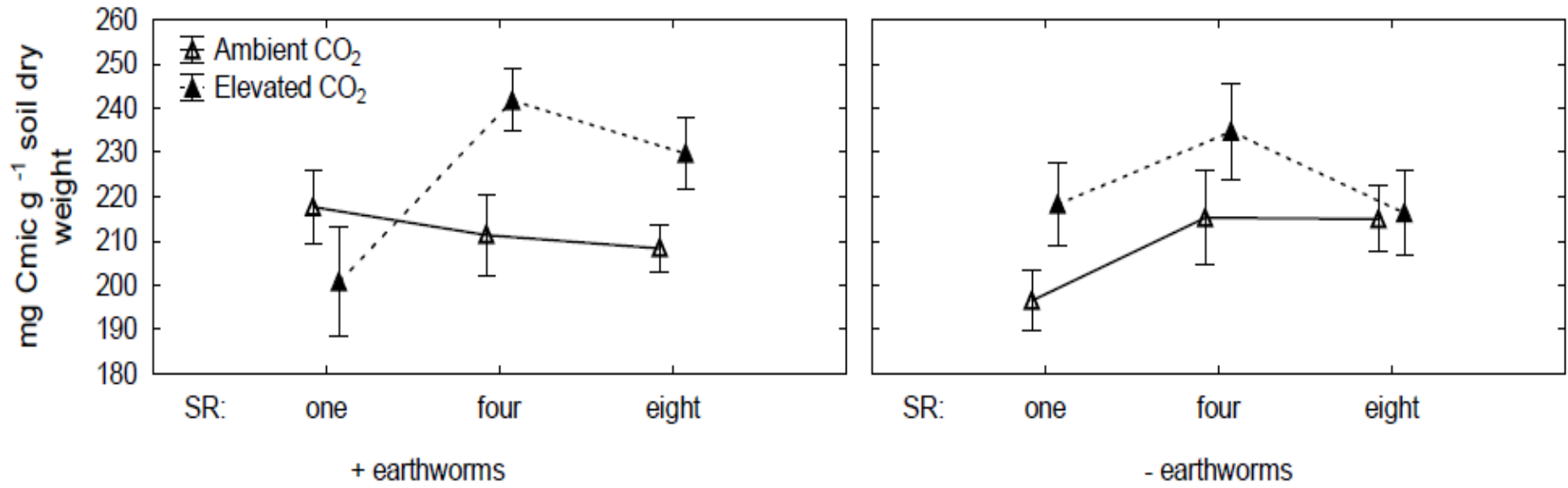
Figure 1

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	Bromus hordeaceus							
2	Monoculture	Cynosurus cristatus							
3	Monoculture	Achillea millefolium							
4	Monoculture	Dipsacus fullonum							
5	Monoculture	Trifolium repens							
6	Monoculture	Vicia cracca							
7	4-species-mixture	Holcus lanatus	Lolium perenne	Vulpia bromoides	Festuca pratensis				
8	4-species-mixture	Taraxacum officinale	Rumex acetosella	Dipsacus fullonum	Daucus carota				
9	4-species-mixture	Trifolium dubium	Trifolium pratense	Medicago lupulina	Vicia sativa				
10	4-species-mixture	Bromus hordeaceus	Lolium perene	Leontodon hispidus	Vicia sativa				
11	4-species-mixture	Bromus sterilis	Plantago lanceolata	Taraxacum officinale	Lotus corniculatus				
12	4-species-mixture	Alopecurus pratensis	Myosotis arvensis	Trifolium pratense	Lathyrus tuberosus				
13	8-species-mixture	Holcus lanatus	Anthoxanthum odoratum	Festuca pratensis	Alopecurus pratensis	Agrostis capillaris	Festuca rubra	Bromus sterilis	Poa annua
14	8-species-mixture	Galium mollugo	Plantago lanceolata	Matricaria recutita	Daucus carota	Prunella vulgaris	Leontodon hispidus	Myosotis arvensis	Rumex acetosella
15	8-species-mixture	Trifolium dubium	Vicia sativa	Lathyrus pratensis	Vicia cracca	Lotus corniculatus	Medicago sativa	Trifolium medium	Medicago lupulina
16	8-species-mixture	Elytrigia repens	Anthoxanthum odoratum	Festuca rubra	Plantago lanceolata	Prunella vulgaris	Dipsacus fullonum	Medicago lupulina	Lotus corniculatus
17	8-species-mixture	Lolium perene	Bromus sterilis	Achillea millefolium	Matricaria recutita	Leucanthemum vulgare	Vicia sativa	Trifolium medium	Medicago sativa
18	8-species-mixture	Dactylis glomerata	Holcus lanatus	Festuca pratensis	Leucanthemum vulgare	Achillea millefolium	Trifolium dubium	Vicia cracca	Lathyrus tuberosus

Grass
Herb
Legume