

Loss of plant biodiversity eliminates stimulatory effect of elevated CO₂ on earthworm activity in grasslands

John A. Arnone III · Johann G. Zaller ·
Gabriela Hofer · Bernhard Schmid ·
Christian Körner

Received: 31 May 2012 / Accepted: 18 December 2012 / Published online: 8 February 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Earthworms are among the world's most important ecosystem engineers because of their effects on soil fertility and plant productivity. Their dependence on plants for carbon, however, means that any changes in plant community structure or function caused by rising atmospheric CO₂ or loss of plant species diversity could affect earthworm activity, which may feed back on plant communities. Production of surface casts measured during three consecutive years in field experimental plots ($n = 24$, 1.2 m²) planted with local calcareous grassland species that varied in plant species richness (diversity levels: high, 31 species; medium, 12; low, 5) and were exposed to ambient (356 $\mu\text{l CO}_2 \text{ l}^{-1}$) or elevated (600 $\mu\text{l CO}_2 \text{ l}^{-1}$) CO₂ was only consistently

stimulated in high diversity plots exposed to elevated CO₂ (+120 %, 31 spp: 603 \pm 52 under ambient CO₂ vs. 1,325 \pm 204 g cast dwt. m⁻² year⁻¹ under elevated CO₂ in 1996; +77 %, 940 \pm 44 vs. 1,663 \pm 204 g cast dwt. m⁻² year⁻¹ in 1998). Reductions in plant diversity had little effect on cast production in ecosystems maintained at ambient CO₂, but the stimulatory effect of elevated CO₂ on cast production disappeared when plant species diversity was decreased to 12 and 5 species. High diversity plots were also the only communities that included plant species that an earlier field study showed to be among the most responsive to elevated CO₂ and to be most preferred by earthworms to deposit casts near. Further, the +87 % CO₂-induced increase in cast production measured over the 3 years corresponded to a parallel increase in cumulative total nitrogen of 5.7 g N m⁻² and would help explain the large stimulation of aboveground plant biomass production observed in high-diversity communities under elevated CO₂. The results of this study demonstrate how the loss of plant species from communities can alter responses of major soil heterotrophs and consequently ecosystem biogeochemistry.

Communicated by Russell Monson.

J. A. Arnone III (✉)
Division of Earth and Ecosystem Sciences, Desert Research
Institute, 2215 Raggio Parkway, Reno, NV 89512, USA
e-mail: Jay.Arnone@dri.edu

J. A. Arnone III · J. G. Zaller · G. Hofer · B. Schmid ·
C. Körner
Botanisches Institut, Universität Basel, Schönbeinstrasse 6,
4056 Basel, Switzerland

J. G. Zaller
Institute of Zoology, University of Natural Resources and Life
Sciences (BOKU), Gregor Mendel Strasse 33,
1180 Vienna, Austria

G. Hofer
Forschungsanstalt, Agroscope Reckenholz-Tänikon ART,
Reckenholzstrasse 191, 8046 Zurich, Switzerland

B. Schmid
Institute of Evolutionary Biology and Environmental Studies,
University of Zürich, Winterthurerstrasse 190,
8057 Zurich, Switzerland

Keywords Ecosystem engineers · Earthworm casts · Plant species richness · Calcareous grasslands · Soil nutrient availability · NPP · CO₂ enrichment · Global change · Key species · *Dactylis* · *Carex* · *Anthoxanthum*

Introduction

Earthworms are nearly ubiquitous globally, but are most abundant in grasslands (Lee 1985) and forests (Phillipson et al. 1976; Satchell 1983; Zicsi 1983) where precipitation is sufficient to keep soils moist and where pH and calcium availability are suitable (Lee 1985). As primary and

secondary consumers/decomposers of above- and below-ground plant litter and soil organic matter (Edwards and Lofty 1977), earthworms ultimately depend on plants to meet their carbon needs (Edwards and Bohlen 1997). Earthworms in turn stimulate soil nutrient mineralization—mainly through soil bioturbation and egestion of nutrient-rich casts—that can enhance nutrient bioavailability and plant growth (e.g., Coleman and Crossley 1996; Scheu 2003). Thus, any environmental change that affects the activity of either plants or earthworms will likely alter the behavior of the other. In grasslands, earthworm biomass can exceed 250 g m^{-2} (Edwards and Bohlen 1997; Zaller and Arnone 1997), and earthworms can produce between 1,500 and 4,500 g of casts (dry mass) per square meter annually (Zaller and Arnone 1997; Glasstetter 1991). These casts can contain as much as 40–300 % more nitrogen and up to 400 % more phosphorous than equivalent amounts of adjacent soil (e.g., Aldag and Graff 1975; Lee 1985), and a large fraction of these nutrients are present in plant-available form (Syers et al. 1979).

In calcareous grassland ecosystems typical of those covering large areas of Europe, our earlier work has shown that exposure of native undisturbed plant communities to elevated levels of atmospheric CO_2 —a global experiment currently playing out due to human combustion of fossil fuels (e.g., Keeling et al. 2005; IPCC 2007)—stimulated earthworm surface casting by 35 % (1,633 vs. 2,206 g dry mass $\text{m}^{-2} \text{ year}^{-1}$) and ecosystem N cycling occurring via surface casts by 30 % (69 vs. 89 kg N $\text{ha}^{-1} \text{ year}^{-1}$; Zaller and Arnone 1997). This effect resulted from both increased net ecosystem CO_2 uptake (e.g., Stocker et al. 1997) and carbon supply to earthworms, as well as from improved soil water status (Niklaus and Körner 2004) deriving from reduced plant transpiration (Lauber and Körner 1997) under elevated CO_2 .

Within these same grasslands in northwestern Switzerland (Leadley et al. 1997), we also found that some plant species (*Anthoxanthum odoratum*, *Dactylis glomerata*, *Carex caryophylla*, and *C. flacca*) were spatially more highly associated with earthworm surface casts than were other plant species (Zaller and Arnone 1999b), and that these plant species responded more strongly (more and larger tillers or ramets) to elevated CO_2 than plant species that were less highly associated with casts (Fig. 1). Some of these same graminoid species also showed reductions in leaf stomatal conductance under elevated CO_2 (Lauber and Körner 1997). Thus, plant water savings by individuals of these species appear to have allowed local topsoil microsites to remain moister than microsites near other plant species and thus promote earthworm casting activity. Indeed, greater growth of individual plants located near larger casts, relative to individuals located near smaller casts, in these undisturbed native grassland communities

regardless of atmospheric CO_2 level pointed to a strong nutrient effect of surface casts in these communities. Certainly, all earthworms living in these grasslands, as is true for all plant communities, ultimately depend on carbon supplied by plants. So, results from our earlier studies strongly suggested (1) some level of dependency of major surface-casting earthworm species on the presence and abundance of particular graminoid species, and (2) that interactions between earthworms and various plant species can help structure plant communities and likely co-determine net primary productivity (NPP).

These observations also clearly suggested that the presence of certain graminoid species in these plant communities may be necessary for elevated CO_2 to stimulate ecosystem-wide surface cast production. To further evaluate this co-dependency, we took advantage of an elaborate experiment being conducted at the same time and at the same site in adjacent native, undisturbed grassland plots (Niklaus et al. 2001; Niklaus and Körner 2004). This experiment quantified the effects of reducing plant species diversity—from the full complement of species (31) down to 12 and 5 species—on ecosystem function under conditions of both ambient and elevated levels of atmospheric CO_2 using constructed plant communities. Serendipitously

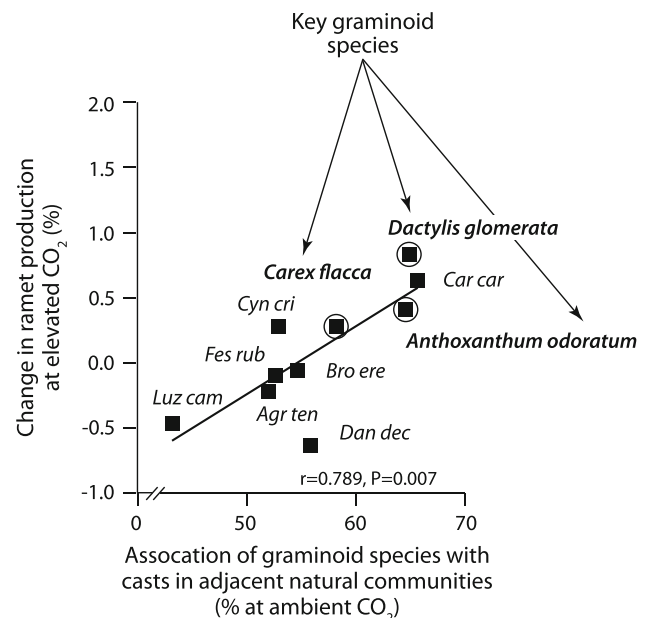


Fig. 1 Positive relationship between the growth response of various plant species previously observed in undisturbed native calcareous grassland ecosystems and the degree to which each species was associated with earthworm surface casts (taken from Zaller and Arnone 1999b) illustrating the strongest CO_2 responses in the graminoid species *Dactylis glomerata*, *Anthoxanthum odoratum*, *Carex flacca*, and *Carex caryophylla* (denoted as *Car car*), the first three of which were planted in the present study's high diversity (31 plant species) plant communities and described here as "key" graminoid species

for the present study, the graminoid species most highly associated with surface casts in native species-rich undisturbed grassland communities were present in constructed communities comprised of 31 plant species but were not included in constructed communities containing 12 and 5 species.

Thus, the objectives of the study presented here were (1) to quantify the extent to which plant species diversity (richness)/plant species identity influenced earthworm activity and cast-mediated nitrogen (N) cycling in designed communities with manipulated numbers of plant species (e.g., Leadley et al. 1997; Niklaus et al. 2001), and (2) to determine if, and how strongly, plant species diversity/identity modulated the effect of ecosystem exposure to elevated CO₂ on earthworm surface cast production and cast-mediated N cycling. Based on both casting responses observed in native undisturbed species-rich grassland plots (Zaller and Arnone 1997) adjacent to the constructed communities examined here, and results from these showing increased soil water contents (Niklaus and Körner 2004) and higher net ecosystem CO₂ exchange (NEE or CO₂ uptake) under elevated CO₂ (Stocker et al. 1999), we expected to see a stimulation of surface casting in the constructed communities exposed to elevated atmospheric CO₂, particularly in plots that were planted with the full complement of species (i.e., high-diversity plots).

Materials and methods

Field site and experimental design

In spring of 1993, 24 replicate semi-natural “designed” experimental calcareous grassland plant communities were constructed on 1.2 m² hexagonally shaped plots arranged along a 50-m-long east-facing hillslope (20° slope) within a species-rich calcareous grassland pasture in the lowland Jura Mountains of northwest Switzerland (Huovinen-Hufschmid and Körner 1998; mean elevation 520 m, 47°33'N, 7°34'E). Annual precipitation averaged 900 mm and annual air temperature ranged between 8.5 and 9.0 °C (1970–1990; Ogermann et al. 1994).

Details of the experimental design, establishment of plant communities with varying diversities/species richness, and technology used to control atmospheric CO₂ levels are described in Leadley et al. (1997) and Niklaus et al. (2001). Briefly, plots were arranged in four blocks from the top of the slope to the bottom, with six plots assigned to each block with one plot representing each plant species diversity × CO₂ treatment combination. We collected data from the ambient and elevated CO₂ plots with Screen Aided CO₂ Control (SACC: open-bottom/open-top chambers; Leadley et al. 1997). Plots with

ambient CO₂ and no SACC were not used for this study. Topsoil (15–20 cm depth, stone-free, loam, neutral pH; Ogermann et al. 1994) from all plots within each block was removed in the spring of 1993, homogenized, and returned to the plots in that block (over the rock-filled subsoil). Most plant species used to establish communities were propagated from seeds collected at the site. Some species were vegetatively propagated using local material.

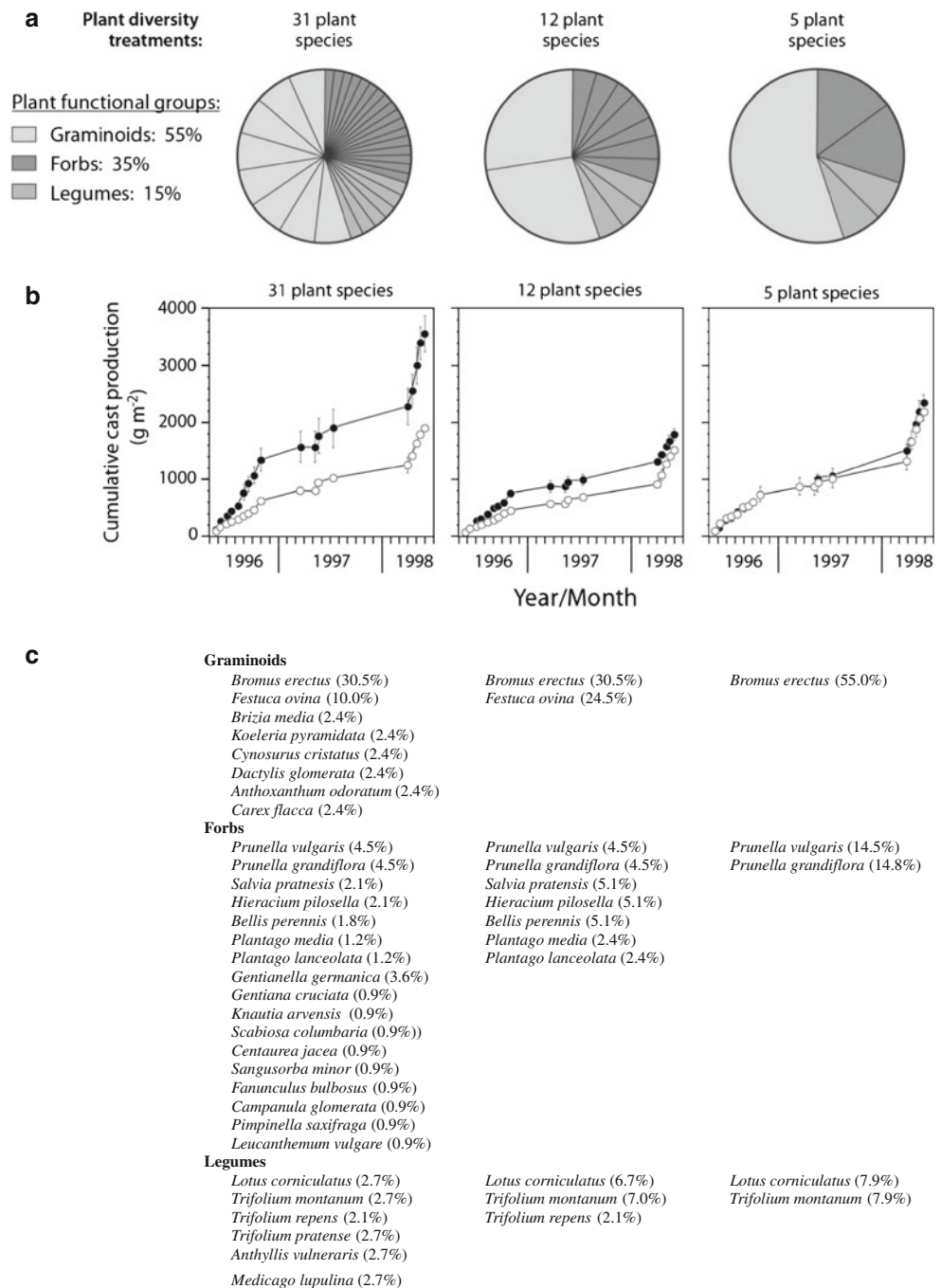
Communities were constructed by planting individuals of 31, 12, or 5 species on a hexagonal grid with a distance of 3.8 cm between individuals (590 m⁻²). Representation of each of the three functional groups—graminoids (55 % of all individuals planted), non-legume forbs (30 %), and legumes (15 %)—was held constant across all plots, to mimic the situation in adjacent undisturbed natural plant communities, with only the number of species in each functional group varying (Fig. 2a, c). Communities planted with 31 species were designed to represent the diversity/richness level of the adjacent native undisturbed grassland communities. In late winter 1994, we began to continuously expose half of the plots to elevated levels of atmospheric CO₂ (600 μl CO₂ l⁻¹) and half to ambient CO₂ levels (356 μl CO₂ l⁻¹; actual measured concentration; Niklaus et al. 2001) using SACC technology. CO₂ treatments were continued until mid-June 1998.

Surface cast production and cast nitrogen content

The main surface-casting earthworm species we observed in these semi-natural ecosystems was *Nicodrilus longus* (Zaller and Arnone 1999a). Including two other anecic species these large-bodied surface casting species accounted for 35 % of all earthworm biomass censused in October 1996. We observed a total of nine earthworm species (Zaller and Arnone 1999a), with worm communities consisting of four small-bodied endogeic species (*Octolasion cyaneum*, *Allolobophora rosea*, *A. chlorotica*, *Nicodrilus caliginosus*), two small-bodied epigeic species (*Lumbricus castaneus*, *Dendrobaena mammalis*), and three large-bodied vertically boring anecic species (*N. longus*, *L. terrestris*, *N. nocturnus*). Six of these species were found in all communities, with the surface caster *N. longus* representing the main cast mass producer in all communities (Zaller and Arnone 1999a). Thus, quantification of surface cast production represents most of the earthworm engineering activity in these constructed grasslands, as was the case in the adjacent undisturbed grassland (Zaller and Arnone 1997).

Beginning in May 1996, during the last 3 years of the 5-year study (Leadley et al. 1997; Niklaus et al. 2001), we measured the mass of newly produced earthworm surface casts (since the last sampling date) in a permanently marked, but randomly selected, 25 × 25 cm area within

Fig. 2 **a** Illustration of the design of plant diversity treatments showing constant representation of plant functional groups—graminoids, forbs, and legumes—and decreases in plant species richness within each of these groups to achieve progressive 60 % reductions in species diversity detailed in the species lists below the diagrams. **b** Time courses of cumulative earthworm surface cast production during observation periods within the 3 years of the study showing, within each of the diversity-treatment panels, differences between ecosystems continuously exposed to ambient (*open circles*) and elevated atmospheric CO₂ (*filled circles*). Percentages given in parentheses after species names indicate the proportion of the total number of individuals planted in each community



each 1.2 m² hexagonally shaped experimental plot every 7–14 days during periods when soil conditions allowed deposition of casts on the soil surface (moist and non-frozen topsoil layer; see Zaller and Arnone 1997). During summer months, earthworms typically aestivate in response to drier soils caused by reductions in precipitation and increases in evapotranspiration resulting from higher summertime air temperatures. Shallow topsoils (15–20 cm deep) that contain >80 % of all root systems (Arnone et al. 2000) had relatively low water storage capacity and dried

fairly quickly once summer began. We measured newly produced cast mass monthly during periods of low cast production when topsoils were dry or frozen. In late 1997, we were unable to measure cast mass production. Termination of the experiment after the 1998 growing season (Niklaus et al. 2001) also precluded quantification of cast production for the entire year. Consequently, cast production measured in 1997 and 1998 was underestimated. However, assessment of relative treatment effects remained possible. We did not spatially map cast production as we

were able to do in the previous study in undisturbed native grassland ecosystems (Zaller and Arnone 1997).

To estimate the total amount of N that was made available through surface-casting activity during the 3 years of observation, we multiplied the total mass of casts produced on each plot during the 3 years by the cast total-N concentration measured in May 1997 (the only time cast total-N was measured, using a LECO TruSpec CN dry combustion analyzer (LECO, St. Joseph, Michigan, USA). Since cast total-N concentrations did not differ among treatments, we used the May 1997 average value calculated across all treatments (i.e., 3.47 mg N g⁻¹ cast dry mass) to calculate the 3 year sum of cast N production. We also found no differences in cast total-N concentration on experimental plots in adjacent undisturbed grassland ecosystems exposed to ambient and elevated CO₂ (4.35 mg N g⁻¹ cast dry mass; Zaller and Arnone 1997).

Statistical analyses

We analyzed data collected during the 3-year observation period in several ways, using Stata 9.0 (StataCorp, College Station, TX, USA); however, in all analyses, we used the plot (one from every treatment in each of the four blocks) as the experimental unit/replicate—i.e., randomized complete block design). To analyze the effects of CO₂ and diversity treatments on total annual surface cast production, we first used the sum of all casts produced on each plot in each calendar year in a three-way analysis of variance (ANOVA) with CO₂ (two levels), diversity (three levels), year (3 years), and their interactions as sources of variation. Because the block effect was never statistically significant ($P > 0.05$), this factor was removed from all ANOVAs. When necessary, data were log-transformed prior to ANOVA to ensure homogeneity of variance among data from all treatment combinations. CO₂ and diversity effects were tested against plot variability. Effects of interaction terms were tested against residual error terms. A priori contrast tests were used to evaluate statistical significance among treatment means. The effect of CO₂ treatment on cumulative cast production within each diversity treatment was also analyzed using repeated measures ANOVA (von Ende 1993). These analyses included as dependent variables (1) annual sums of cumulative cast production over the 3 years of observation, (2) cumulative cast production calculated over individual sampling dates over the 3 years of observation, and (3) cumulative cast production calculated over individual sampling dates within each year of observation. To evaluate the relationships between mean treatment aboveground net primary productivity (ANPP) and mean treatment cast production, we used simple linear regression analysis. Effects with a $P < 0.05$ were considered statistically significant.

Results

Earthworm cast production observed across all plant diversity levels and both atmospheric CO₂ treatments varied from year to year, with the highest production observed in spring and early summer of 1998 and lowest production in spring and early summer of 1997 (Tables 1, 2; Fig. 2b). Under ambient CO₂ during the first 2 years of observation (1996, 1997), plant diversity had no effect on cumulative surface cast production ($P_{\text{diversity at amb CO}_2 \text{ 1996}} = 0.0802$, $P_{\text{diversity at amb CO}_2 \text{ 1997}} = 0.1660$). During the last year (1998) of the study, however, reducing diversity from 12 to 5 species appeared to stimulate cast production (+37 %; $P_{\text{amb CO}_2-12 \text{ vs. } 5 \text{ spp}} = 0.0004$; $P_{\text{diversity at amb CO}_2 \text{ 1998}} = 0.0006$) while reducing plant diversity from 31 to 12 species had no effect on cast production ($P_{\text{amb CO}_2-31 \text{ vs. } 12 \text{ spp}} = 0.1909$). This final year's effect not only resulted in a corresponding effect on cumulative cast production observed across all 3 years at ambient CO₂ ($P_{\text{diversity at amb CO}_2 \text{ 1996–1998}} = 0.0138$; +45 %, $P_{\text{amb CO}_2-12 \text{ vs. } 5 \text{ spp}} \text{ 1996–1998} = 0.0144$) but also in a slight (+16 %) cumulative 3-year stimulation of cast production in low diversity plots relative to that observed in the high diversity plots ($P_{\text{amb CO}_2-31 \text{ vs. } 5 \text{ spp}} = 0.0007$).

Continuous exposure to elevated atmospheric CO₂, however, greatly modulated these diversity-induced patterns found under ambient CO₂ concentration (Tables 1, 2; Fig. 2b). Under elevated CO₂, plant species diversity influenced total observed cast production in each of the 3 years ($P_{\text{diversity at elev CO}_2 \text{ 1996}} = 0.0211$; $P_{\text{diversity at elev CO}_2 \text{ 1997}} = 0.0487$; $P_{\text{diversity at elev CO}_2 \text{ 1998}} = 0.0029$) with the diversity effect due mainly to a large stimulation in cast production in high diversity ecosystems relative to that measured in medium and low diversity ecosystems ($P_{\text{elev CO}_2-31 \text{ vs. } 12 \text{ spp}} \text{ 1996} = 0.0357$, $P_{\text{elev CO}_2-12 \text{ vs. } 5 \text{ spp}} \text{ 1996} = 0.8656$, $P_{\text{elev CO}_2-31 \text{ vs. } 5 \text{ spp}} \text{ 1996} = 0.0065$; $P_{\text{elev CO}_2-31 \text{ vs. } 12 \text{ spp}} \text{ 1997} = 0.0365$, $P_{\text{elev CO}_2-12 \text{ vs. } 5 \text{ spp}} \text{ 1997} = 0.1661$, $P_{\text{elev CO}_2-31 \text{ vs. } 5 \text{ spp}} \text{ 1997} = 0.0677$). However, in 1998, and indeed across all 3 years ($P_{\text{diversity at elev CO}_2 \text{ 1996–1998}} = 0.0009$), the diversity effect under elevated CO₂ was explained by differences in cast production among plots at all plant diversity levels ($P_{\text{elev CO}_2-31 \text{ vs. } 12 \text{ spp}} \text{ 1998} = 0.0058$, $P_{\text{elev CO}_2-12 \text{ vs. } 5 \text{ spp}} \text{ 1998} = 0.0176$, $P_{\text{elev CO}_2-31 \text{ vs. } 5 \text{ spp}} \text{ 1998} = 0.0110$).

The different effects of plant diversity under the two CO₂ levels were reflected in strong statistical interactions between plant species diversity and atmospheric CO₂, with interactions differing between years of observation (Figs. 2b, 3; Table 2). Elevated CO₂ stimulated surface cast production in 1996 in high-diversity plant communities by +120 % and by almost 65 % in communities with 12 species. However, elevated CO₂ did not stimulate cast

Table 1 Effects of plant species diversity and atmospheric CO₂ on earthworm surface cast production during observation periods in 1996, 1997, and 1998, and cast total-N concentration measured in May 1997, observed in planted calcareous grassland communities in the lowland Jura Mountains of northwestern Switzerland (values are expressed as mean ± SE, *n* = 4 plots)

Cast production				
Year/Plant diversity	CO ₂ level (μl CO ₂ l ⁻¹)		Mean difference (%)	<i>P</i> value
	Amb CO ₂ (g cast m ⁻² year ⁻¹)	Elev CO ₂ (g cast m ⁻² year ⁻¹)		
1996				
High (31 spp)	603 ± 52	1325 ± 204	+120	0.0069
Medium (12 spp)	460 ± 49	752 ± 83	+64	0.0212
Low (5 spp)	739 ± 126	728 ± 73		0.9626
1997				
High	404 ± 47	561 ± 126		0.3824
Medium	222 ± 48	237 ± 38		0.7231
Low	277 ± 60	338 ± 50		0.4464
1998				
High	940 ± 44	1663 ± 204	+77	0.0109
Medium	859 ± 26	691 ± 121		0.2163
Low	1180 ± 27	1294 ± 45		0.0711
Cast total-N concentration (%)				
1997 May				
High	0.343 ± 0.016	0.350 ± 0.019		0.7970
Medium	0.353 ± 0.017	0.357 ± 0.021		0.9080
Low	0.353 ± 0.023	0.328 ± 0.004		0.3185

production in low-diversity communities. The stimulatory effect of elevated CO₂ disappeared in 1997 and returned in 1998 in high diversity communities with a smaller stimulation (+77 % in communities with high species diversity, no stimulation at the two lower diversity levels).

Neither elevated CO₂ nor plant diversity affected total-N concentrations of casts (Tables 1, 2). Therefore, treatment effects on the calculated cumulative amount of N egested during the 3 years (Fig. 3 inset) of observation were identical to those described above for cumulative cast mass production (Fig. 3; Table 2). When viewed across all treatments, mean total surface cast production summed over all 3 years was positively correlated with mean total aboveground net primary productivity (ANPP, i.e., plant biomass production) summed over the 3 years (Fig. 4; ANPP data from Niklaus et al. 2001). This relationship between ANPP and cumulative surface cast production generally also held true for individual years (Fig. 4b).

Environmental conditions in all 3 years of observation were suitable for surface casting earthworms to remain active in all experimental plots (Fig. 5). Average air

temperature, precipitation, and daytime photon flux density (PFD) conditions measured during the first 6 months of each year suggested that 1996 was relatively cool and dry (6.6 °C, 343 mm, 605 mol m⁻² month⁻¹), 1997 was warmer (7.8 °C) with moderately more precipitation (400 mm) but with the highest PFD (669 mol m⁻² month⁻¹), and 1998 was warmer still (8.6 °C) with the most precipitation (456 mm) and PFDs (607 mol m⁻² month⁻¹) similar to those seen in 1996. Thus, spring and early summer of 1998 appeared to have had the best conditions for earthworm casting activity (warm, moist, low PFD; cf. Lee 1985). Water contents measured in our experimental plots and reported in Niklaus et al. (2007) also indicated suitable topsoil moisture conditions during springtime and early summer in all plots in all years. They observed no effects of plant species diversity on gravimetric soil WC of the top 30 cm but a significant and sustained increase in plots maintained under elevated CO₂ (mean growing season WC of 28 vs. 26 % in control plots). Thus, topsoils of plots maintained at high CO₂ remained moister and moister longer into dry summer periods and soil WC recovered sooner in early fall. Soil WC was not affected by interactions between plant species diversity and atmospheric CO₂ level (Niklaus et al. 2007).

Discussion

Interannual variability in climatic (Fig. 5) and soil moisture conditions (Niklaus et al. 2007) generally accounted for overall year–year differences in earthworm surface cast production within treatments, especially when production was adjusted for the length of the observation period (Fig. 4b). The production rates were also comparable to rates measured at this site in native undisturbed plots (Zaller and Arnone 1997). Reductions in the size (biomass and density) of earthworm communities observed in 1996 with decreasing number (or type) of plant species within communities, as well as the lack of an effect of elevated CO₂ on earthworm community biomass or density (Zaller and Arnone 1999a), indicate that the changes in surface casting activity we observed were primarily due to changes in the specific activity of the large surface-casting species (Fig. 2b). The relatively few effects of plant species diversity we observed on cast production under ambient CO₂ (Fig. 2b; Table 1) were unexpected because the three “key” graminoid species, *Anthoxanthum odoratum*, *Dactylis glomerata* and *Carex flacca*, favored by vertical-boring surface-casting anecic earthworms, were present in all high-diversity communities. In contrast, Spehn et al. (2000) observed significant reductions in earthworm activity with large decreases in plant species diversity in planted grasslands under ambient CO₂; however, these

Table 2 Results (*P* values) of two-way, three-way, and repeated measures ANOVAs of the independent variables cumulative surface cast production, measured during the 1996 (spring through fall), 1997 and 1998 (spring and summer) growing seasons as a function of plantspecies diversity and atmospheric CO₂ concentration; as well as cast total-N concentration measured in May 1997 and 3-year cumulative cast mass and cast N production

Total annual cast production (using sum of cast production occurring in each year in each plot)						
CO ₂	0.0106	*				
Diversity	0.0006	***				
Year	0.0007	***				
CO ₂ × diversity	0.2204					
CO ₂ × year	0.2680					
Diversity × year	0.2920					
CO ₂ × diversity × year	0.0209	*				
Total annual cast production (using sum of cast production occurring in each year in each plot)						
	1996		1997		1998	
CO ₂	0.0087	**	0.2550		0.4200	
Diversity	0.0546	(*)	0.0134	*	0.0205	*
CO ₂ × diversity	0.1103		0.6999		0.1706	
Cumulative cast production (using individual sampling dates, repeated measures)						
	High diversity		Medium diversity		Low diversity	
All 3 years						
CO ₂	0.0145	*	0.0176	*	0.8545	
Julian date 1996	<0.0001	***	<0.0001	***	<0.0001	***
CO ₂ × Julian date	<0.0001	***	0.0002	***	0.9222	
1996						
CO ₂	0.0482	*	0.0652	(*)	0.6150	
Julian date 1996	<0.0001	***	<0.0001	***	<0.0001	***
CO ₂ × Julian date	0.0032	**	0.6068		0.3302	
1996 + 1997						
CO ₂	0.0231	*	0.0461	*	0.7774	
Julian date 1996	<0.0001	***	<0.0001	***	<0.0001	***
CO ₂ × Julian date	0.0129	*	0.5141		0.6201	
1996 + 1997 + 1998						
CO ₂	0.0058	**	0.0660	(*)	0.6264	
Julian date 1996	<0.0001	***	<0.0001	***	<0.0001	***
CO ₂ × Julian date	0.8617		0.0003	**	0.1555	
Cast total-N concentration May 1997						
CO ₂	0.7295					
Diversity	0.7315					
CO ₂ × diversity	0.6316					
Cumulative 3-year cast production and cumulative N production (sum of all 3 years)						
CO ₂	0.0056	**				
Diversity	0.0111	*				
CO ₂ × diversity	0.0139	*				

The CO₂ effect was tested against the plot-within-CO₂ error term (*df* 1, 6) and the diversity effect against the plot-within-diversity error term (*df* 2, 6). The interactive effect (CO₂ × diversity) was tested against the residual error term (*df* 2, 6)

Significance levels: * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001

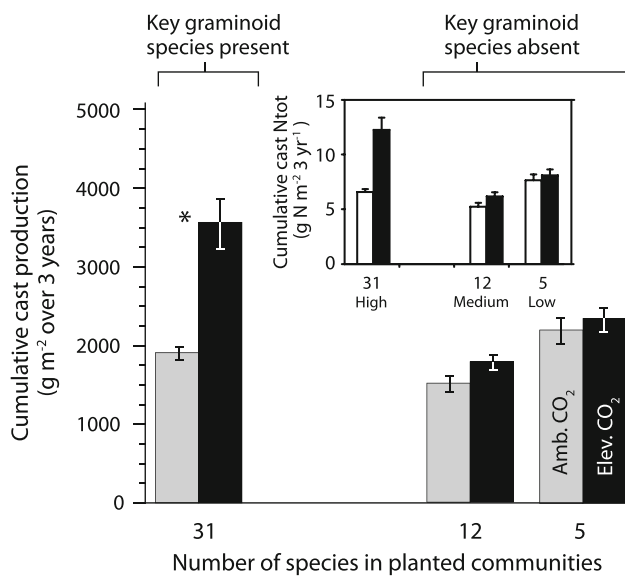


Fig. 3 Mean (\pm SE) cumulative surface cast production summed over the 3 years of measurements, and mean (\pm SE) estimated cumulative amount of total N in these casts (*inset*), expressed as a function of plant community species diversity and atmospheric CO₂ level ($n = 4$ experimental plots equipped with SACC chambers for a total of 24 plots)

decreases were due to large reductions in earthworm population density and biomass. The significantly higher cumulative cast production that we measured in low-diversity plots relative to that measured in medium-diversity plots may have to do with the presence of more favorable microsite conditions for surface-casting earthworms in low-diversity plots (aside from the plant variables measured; Niklaus et al. 2001), or possibly to unknown net inhibitory effects on surface cast production exerted by plant species comprising the medium-diversity communities. Also surprising in our study was that small but very consistent 1.5–1.9 % higher soil water content measured in plots exposed to elevated CO₂ during the last 3 years of the study (1996–1998; Niklaus et al. 2007) did

not lead to a parallel uniform increase in cast production under elevated CO₂. However, the large CO₂ stimulation of cast production observed in high-diversity communities, only moderate 1 year (1996) effects seen in medium-diversity communities, and lack of CO₂ effects on cast production in low-diversity communities clearly demonstrate that potential anthropogenic losses of grassland plant species diversity in a world with continually rising anthropogenic CO₂ may profoundly affect the activity of earthworms and earthworm-cast-mediated soil nutrient availability. These effects could then strongly modulate plant community NPP. At the very least, these significant CO₂ \times plant diversity interactions indicated that earthworm activity response to elevated CO₂ depended on the number of plant species present in communities or on the presence of particular plant species.

Analyses of total soil N pools and N pools of annually produced shoot biomass measured during the course of the entire experimental period (1994–1998) in the same experimental plots examined in the present study showed no effects of plant species diversity or exposure to elevated CO₂ (Niklaus et al. 2001). However, our results showing a stimulation of surface cast production—and hence faster cycling of labile forms of nitrogen (cf. Aldag and Graff 1975; Syers et al. 1979; Scheu 1993)—under elevated atmospheric CO₂ only in plots containing the highest diversity of plant species, suggest that cumulative N availability in these plots (Fig. 3 inset) was stimulated through the casting activity of earthworms and earthworm soil bioturbation (e.g., Edwards and Bohlen 1997). Estimates of total cast production and N cycling occurring through surface-casting activity measured in our study are likely below the actual annual totals because sampling was not continuous during the entire year but focused on the most active periods. Some surface cast production occurs throughout the fall and even during the warmer periods of winter and early spring (J. Zaller, unpublished data). Also, if subsurface cast production of endogeic earthworm

Fig. 4 Simple linear regression of mean (\pm SE) treatment cumulative surface cast production **a** summed over the 3 years of observation on corresponding mean (\pm SE) aboveground net primary biomass productivity (ANPP) showing a significant positive relationship ($P_{\text{slope}} = 0.0179$, $r^2 = 0.79$); and **b** for each year separately (1996: $P_{\text{slope}} = 0.18$, $r^2 = 0.40$; 1997: $P_{\text{slope}} = 0.11$, $r^2 = 0.51$; 1998: $P_{\text{slope}} = 0.01$, $r^2 = 0.86$)

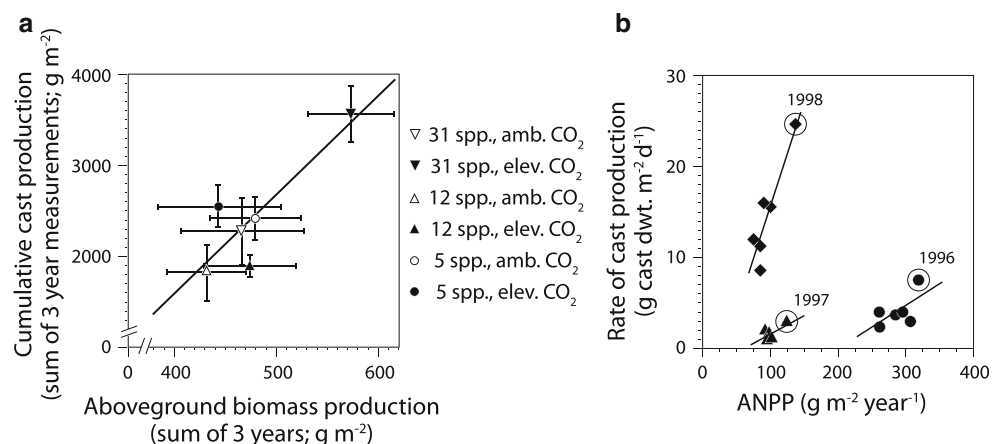
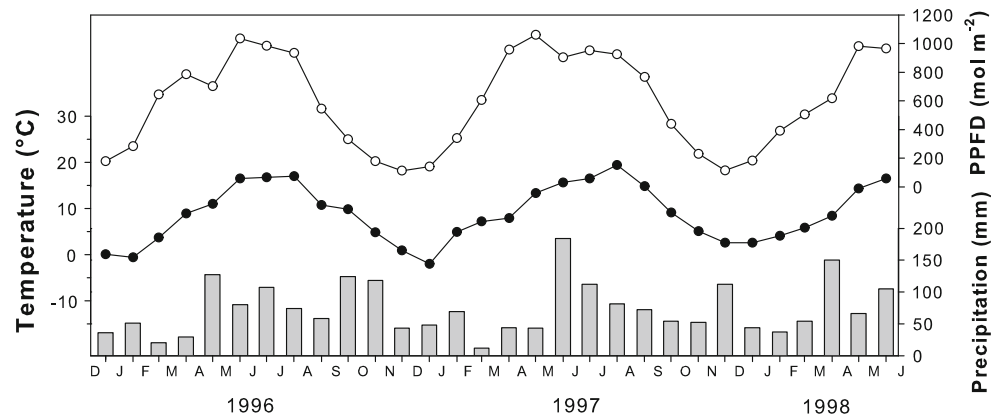


Fig. 5 Monthly (totals, or means in the case of air temperature) environmental conditions at the grassland field site in the lowland Jura Mountains in northwestern Switzerland from the beginning of 1996 to the end of June 1998 when the experiment was terminated (air temperature: filled circles; photosynthetic photon flux density: PPFD open circles; precipitation: shaded bars)



species, which by numbers (not mass) comprised the largest functional group of earthworms in our experimental plots (Zaller and Arnone 1999b), responded to experimental manipulations of CO₂ and plant species diversity or identity in the same fashion as surface casting by anecic species, this group would likely have contributed to responses observed in ANPP.

While we present scatter plots with ANPP on the x-axis, indicating this as the independent variable under consideration and cast production as the dependent variable (Fig. 4), it may be as proper to depict ANPP as depending on earthworm cast production. Clearly, experimental manipulation of plant community species diversity and species composition can directly affect ANPP and qualify it as an independent variable. Moreover, plants within the community can be considered as independent variables because they represent the first-responders to elevated atmospheric CO₂, allowing more moisture to remain in the soil because of reduced transpiration and to inject more carbon into soils via stimulated near-surface root turnover (Arnone et al. 2000). However, plant-mediated stimulation of earthworm cast production under elevated atmospheric CO₂ may quickly feed back to modulate ANPP via enhancement of soil fertility.

The greatest stimulation of plant ANPP observed in plots with the greatest species richness under elevated CO₂ in the first (1996) and last (1998) years of our study may be due to either a general species richness effect itself (Fig. 2) or to the presence of the three “key” graminoid species (*Anthoxanthum odoratum*, *Dactylis glomerata* and *Carex flacca*). Either one of these possibilities, or both, may have created microsite conditions that promoted surface casting of large vertical-boring earthworm species. These conditions might have included: (1) localized and general increases in soil water content in plots maintained at elevated atmospheric CO₂ (Zaller and Arnone 1997, 1999c; Niklaus et al. 2007) resulting from CO₂-induced reductions

in leaf stomatal conductance (Lauber and Körner 1997); (2) greater carbon inputs to upper soil layers in plots kept at high CO₂ due to an upward shift in root production and mortality (“turnover”; Arnone et al. 2000); or (3) overall higher plant carbon supply to earthworms deriving from both higher net ecosystem CO₂ exchange (uptake; Niklaus and Körner 2004) under elevated atmospheric CO₂ and from further enhanced NPP driven by earthworm-induced increases in nutrient cycling rates and stimulated availability of labile plant nutrients (e.g., Syers et al. 1979). Our experimental design did not allow for unequivocal separation of potential pure species-richness effects from species identity effects (i.e., presence or absence of the earthworm-preferred graminoid species; Zaller and Arnone 1999b).

Thus, the results of the study presented here, together with findings from our earlier research in adjacent native intact calcareous grassland plots (Zaller and Arnone 1997; Zaller and Arnone 1999b), indicate that stimulation of earthworm surface-casting activity in ecosystems exposed to elevated atmospheric CO₂ only occurs when the full assemblage of plant species is present or when specific plant species are present in the community (i.e., those most highly associated with surface casts; Zaller and Arnone 1999b). The collective results also highlight that unexpected responses of ecosystem engineers in many terrestrial ecosystems could occur as levels of atmospheric CO₂ continue to rise, and that these responses may be modulated by the composition and diversity of the plant communities they inhabit. Our data also warn of unexpected consequences for the functioning of many other ecosystems where (1) the earthworm species present are not native members of the soil heterotrophic community or where earthworms are themselves not native (Bohlen 2006; Eisenhauer et al. 2012), and (2) ecosystem processes are heavily modulated by key heterotrophic “engineers” (Zaller et al. 2011).

Acknowledgments We thank P. Niklaus and P. Leadley for their assistance in setting up and maintaining the CO₂-enrichment and plant biodiversity treatments, T. Morgan and R. Blank for analyzing total N in casts, and J. Larsen and L. Wable for their help in producing the graphics used in the figures of this paper. Financial support for this research was provided by the Swiss National Science Foundation (NF) through grants to Arnone (NF 3100-042401.94/1) and Körner, Schmid and Arnone (NF-SPPU 5001-035214).

References

- Aldag R, Graff O (1975) N-Fractionen in Regenwurmlosung und deren Ursprungsböden. *Pedobiologia* 15:151–153
- Arnone JA III, Zaller JG, Spehn EM, Niklaus PA, Wells CE, Körner Ch (2000) Dynamics of root systems in intact native grasslands: effects of elevated atmospheric CO₂. *New Phytol* 147:73–86
- Bohlen PJ (2006) Biological invasions: linking the aboveground and belowground consequences. *Appl Soil Ecol* 32:1–5
- Coleman DC, Crossley DA Jr (1996) *Fundamentals of Soil Ecology*. Academic, San Diego
- Edwards CA, Bohlen PJ (1997) *Biology and Ecology of Earthworms*. Chapman and Hall, London, pp 55–180
- Edwards CA, Lofty R (1977) *The Biology of Earthworms*, 2nd edn. Chapman and Hall, London
- Eisenhauer N, Fisichelli NA, Frelich LE, Reich PB (2012) Interactive effects of global warming and “global worming” on the initial establishment of native and exotic herbaceous plant species. *Oikos* 121:1121–1133
- Glasstetter M (1991) Die Bodenfauna und ihre Beziehungen zum Nährstoffhaushalt in Geosystemen des Tafel- und Faltenjura (Nordwestschweiz). *Physiogeographica* 15:1–231
- Huovinen-Hufschmid C, Körner Ch (1998) Microscale patterns of species distribution and biomass in calcareous grassland. *Bot Helv* 108:69–83
- Intergovernmental Panel on Climate Change (2007) *Climate Change: the physical science basis—contribution of working group I to the fourth assessment report of the Intergovernmental panel on climate change*. In: Solomon S et al. (eds) *Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, New York
- Keeling CD, Piper SC, Bacastow RB, Wahlen M, Whorf TP, Heimann M, Meijer HA (2005) Atmospheric CO₂ and ¹³CO₂ exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications. In: Ehleringer JR, Cerling TE, Dearing MD (eds) *A History of Atmospheric CO₂ and its Effects on Plants, Animals, and Ecosystems*. Springer, New York, pp 83–113
- Lauber W, Körner Ch (1997) In situ stomatal responses to long term CO₂ enrichment in calcareous grassland plants. *Acta Oecol* 18:221–229
- Leadley PW, Niklaus P, Stocker R, Körner Ch (1997) Screen aided CO₂ control (SACC): a middle ground between FACE and open top chamber. *Acta Oecol* 18:207–219
- Lee KE (1985) *Earthworms: their ecology and relationships with soils and land use*. Academic, Sydney
- Niklaus PA, Körner Ch (2004) Synthesis of a six-year study of calcareous grassland responses to in situ CO₂ enrichment. *Ecol Monogr* 74:491–511
- Niklaus PA, Wohlfender M, Siegwolf R, Körner Ch (2001) Effects of six years atmospheric CO₂ enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant Soil* 233:189–202
- Niklaus PA, Alpehi J, Kampichler C, Kandeler E, Körner Ch, Tscherko D, Wohlfender M (2007) Interactive effects of plant species diversity and elevated CO₂ on soil biota and nutrient cycling. *Ecology* 88:3153–3163
- Ogermann P, Spycher B, Schaub D, Sollberger R (1994) Die Landschaftsstruktur im Raum Nenzlingen—geökologisch gesehen. *Regio Basiliensis* 35:91–100
- Phillipson J, Abel R, Steel J, Woodell SRJ (1976) Earthworms and the factors governing their distribution in an English beech-wood. *Pedobiologia* 16:258–285
- Satchell JE (ed) (1983) *Earthworm Ecology: from Darwin to Vermiculture*. Chapman and Hall, London
- Scheu S (1993) Analysis of the microbial nutrient status in soil microcompartments: earthworm feces from a basalt limestone gradient. *Geoderma* 56:575–586
- Scheu S (2003) Effects of earthworms on plant growth: patterns and perspectives. *Pedobiologia* 47:846–856
- Spehn EM, Joshi J, Alpehi J, Schmid B, Korner Ch (2000) Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant Soil* 224:217–230
- Stocker R, Leadley PW, Körner C (1997) Carbon and water fluxes in a calcareous grassland under elevated CO₂. *Funct Ecol* 11:222–230
- Stocker R, Körner Ch, Schmid B, Niklaus PA, Leadley PW (1999) A field study of the effects of elevated CO₂ and plant species diversity on ecosystem-level gas exchange in a planted calcareous grassland. *Glob Change Biol* 5:95–105
- Syers JK, Sharpley AN, Keeney DR (1979) Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem. *Soil Biol Biochem* 11:181–185
- von Ende CN (1993) Repeated-measures analysis: growth and other time-dependent measures. In: Scheiner SM, Gurevitch J (eds) *Design and analysis of ecological experiments*. Chapman and Hall, New York, pp 113–137
- Zaller JG, Arnone JA III (1997) Activity of surface-casting earthworms in a calcareous grassland under elevated atmospheric CO₂. *Oecologia* 111:249–254
- Zaller JG, Arnone JA III (1999a) Earthworm responses to plant species’ loss and elevated CO₂ in calcareous grassland. *Plant Soil* 208:1–8
- Zaller JG, Arnone JA III (1999b) Interactions between earthworm casts and plant species in a calcareous grassland under elevated CO₂. *Ecology* 80:873–881
- Zaller JG, Arnone JA III (1999c) Earthworm and soil moisture effects on the productivity and structure of grassland communities. *Soil Biol Biochem* 31:517–523
- Zaller JG, Heigl F, Grabaier A, Lichtenegger C, Piller K, Allabashi R, Frank T, Drapela T (2011) Earthworm-mycorrhiza interactions can affect the diversity, structure and functioning of establishing model grassland communities. *PLoS ONE* 6:1–9
- Zicsi A (1983) Earthworm ecology in deciduous forests in central and southeast Europe. In: Satchell JE (ed) *Earthworm Ecology: from Darwin to Vermiculture*. Chapman and Hall, London, pp 171–177