Predatory beetles facilitate plant growth by driving earthworms to lower soil layers

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Summary

1. Theory suggests that predators of soil-improving, plant-facilitating detritivores (e.g. earthworms) should suppress plant growth via a negative tri-trophic cascade, but the empirical evidence is still largely lacking.

2. We tested this prediction in an alpine meadow on the Tibetan Plateau by manipulating predatory beetles (presence/absence) and quantifying (i) direct effects on the density and behaviour of earthworms; and (ii) indirect effects on soil properties and above-ground plant biomass.

3. In the absence of predators, earthworms improved soil properties, but did not significantly affect plant biomass. Surprisingly, the presence of predators strengthened the positive effect of earthworms on soil properties leading to the emergence of a positive indirect effect of predators on plant biomass.

4. We attribute this counterintuitive result to: (i) the inability of predators to suppress overall earthworm density; and (ii) the predator-induced earthworm habitat shift from the upper to lower soil layer that enhanced their soil-modifying, plant-facilitating, effects.

5. Our results reveal that plant-level consequences of predators as transmitted through detritivores can hinge on behaviour-mediated indirect interactions that have the potential to overturn predictions based solely on trophic interactions.

6. This work calls for a closer examination of the effects of predators in detritus food webs and the development of spatially explicit theory capable of predicting the occurrence and consequences of predator-induced detritivore behavioural shifts.

Key-words: alpine meadow, beetle, behaviour-mediated indirect interaction, detritus, earthworm, food chain, trophic cascade

Introduction

Understanding the functional role of predators in natural and managed ecosystems has been a longstanding goal in ecology (Hairston, Smith & Slobodkin 1960; Oksanen et al. 1981; Romero & Koricheva 2011) and is of growing pertinence in the light of anthropogenic predator depletion and resultant ‘trophic downgrading’ of food webs (sensu Estes et al. 2011). It is now widely recognized that predators can indirectly regulate plant growth, plant biomass and associated ecosystem functions by triggering trophic cascades (reviewed by Pace et al. 1999; Schmitz, Hambäck & Beckerman 2000). Classic trophic cascade theory (Hairston, Smith & Slobodkin 1960; Oksanen et al. 1981) and case studies (e.g. Estes, Smith & Palisano 1978; Visser, Muller-Landau & Wright 2011) highlight the plant-facilitating indirect effects of predators driven by suppression of herbivore density; a so-called ‘green-world’ role of predators (sensu Hairston, Smith & Slobodkin 1960). This theoretical framework should be extendable beyond the traditional emphasis on predator–herbivore–plant chains, to include chains where intermediate species themselves interact positively (non-trophically) with plants (Pearson 2010), but this has rarely been attempted.

Detritivores provide an opportunity to extend and test basic trophic cascade theory; they typically have positive non-trophic effects on plants through their mediation of nutrient release from the detritus pool (Lee 1985; Dominguez, Bohlen & Parmelee 2004) and are often trophically linked to predators. Predator suppression of detritivore – as opposed to herbivore – density (e.g., Srivastava et al. 2009) should straightforwardly switch the sign of indirect predator effects to negative; i.e. predator
reduction of plant growth and biomass (dubbed a ‘brown-world’ role of predators; Allison 2006). In support of this prediction, a brown-world role of predators was recently demonstrated in an alpine meadow, where predators indirectly suppressed plant growth by limiting the densities and activities of coprophagous beetles (detritivores), subsequently decreasing dung decomposition rates and soil nutrient concentrations (Wu et al. 2011). Nevertheless, such cascading effects involving both trophic and non-trophic interactions remain relatively poorly studied particularly in predator–detritivore–plant systems (but see Wardle et al. 2012).

Trophic cascades, whether transmitted through herbivores or detritivores, have been traditionally conceptualized as driven solely by predator suppression of prey density (Hairston, Smith & Slobodkin 1960; Oksanen et al. 1981; Wu et al. 2011; i.e. density-mediated indirect interactions; DMIIs). However, predators can also indirectly affect plants by triggering risk-reducing changes in the traits (i.e. morphology, phenology, physiology or behaviour) of their prey (Beckerman, Uriarte & Schmitz 1997; Trussell, Ewanchuk & Matassa 2006; Maron & Pearson 2011; i.e. trait-mediated indirect interactions; TMIIs). During the last 15 years, TMIIs have been increasingly recognized as comparable in importance to DMIIs in green-world cascades and are now viewed as integral to understanding the indirect effects of predators on plants (see Duffy 2002; Werner & Peacor 2003; Schmitz, Krivan & Ovadia 2004; Schmitz 2008). Behavioural traits of prey can be highly labile in the face of predation risk and therefore frequently serve as a conduit of TMIIs. Chronic habitats shifting is one commonly observed prey behavioural response to predation risk, whereby prey animals may temporally and spatially avoid encountering predators (Edmunds 1974; Abrams 1984). In tri-trophic predator–herbivore–plant systems, these shifts are often concomitant with reduced feeding rates of herbivores and the effect sign of TMIIs measured across the plant community (notwithstanding differential local effects and possible plant release in risky habitats) has been found to be mostly positive and therefore reinforce DMIIs (reviewed by Schmitz, Krivan & Ovadia 2004; but see Griffin et al. 2011). In tri-trophic predator–detritivore–plant systems, similar predator-induced habitat shifts and suppression of detritivore activities may also occur, concomitant with a general weakening of the positive effect of detritivores on plants and the emergence of negative predator-plant TMIIs. However, while a long period of empirical attention has revealed the widespread importance of TMIIs in green-world cascades, the possible contribution of TMIIs to so-called brown-world cascades has not yet been assessed.

In the study presented here, we aimed to further test the hypothesized plant-suppressing, ‘brown-world’ role of predators as transmitted through detritivores. We additionally aimed to examine the potential for trait-mediated indirect interactions to modify these predicted negative indirect interactions. Specifically, we tested the effects of a predatory beetle species on the density and spatial habitat use of two of its prey species (earthworms), as well as soil properties and plant growth rate. Because earthworms, as detritivores and soil engineers, are more often known to facilitate and not suppress plant growth by enhancing nutrient availability, we followed established theory of tri-level interaction chains to predict that predatory beetles would indirectly reduce plant growth rates by consuming earthworms.

Materials and methods

STUDY SITE AND NATURAL HISTORY

Our study was conducted in the Hongyuan Alpine Meadow Ecosystem Research Station (Chinese Academy of Sciences), located in Sichuan province in the eastern Qinghai-Tibetan Plateau (32°48′ N, 102°33′ E). The altitude is 3500 m. The climate is cold, continental and characterized by a short and cool spring, summer and autumn and a long winter. In the period of 1961–2008, as recorded by Hongyuan County Climate Station (located 5 km from the study site), the annual mean temperature was 0.9 °C, with maximum and minimum monthly means of 10.9 °C and −10.3 °C in July and January respectively. The annual mean precipitation was 690 mm (80% of which occurs between May and August).

Vegetative cover of the meadow is over 90%, and average maximum plant height was ca. 30 cm at the time of our study. Vegetation was dominated by sedges such as Kobresia uncinoides and Kobresia pygmaea and grasses such as Elymus nutans and Agrostis hagoniana. Forb species, including, Saussurea nigriscens, Galium boreale, Polygonum macrophyllum, Anemone trullifolia var. linearis and Thalictrum alpinum were also abundant. The meadow has been principally grazed by yaks (Bos graminis) for decades (Xiang et al. 2009). Possibly because of high density of cattle dung (5900 pats per hectare as the highest recorded before; Wu & Sun 2010), the community includes a diverse detritivore assemblage, including beetles, flies, ants, earthworms and their associated predators (e.g. spiders, centipedes and predatory beetles; Wu & Sun 2010).

At our study site, there are two earthworm species, Aporrectodea nocturna and Phereetima aspergillum. Whether these species are native or non-native is currently unknown due to poor historical records for the region; however, both are known to have been active in the study region for about one decade. The former species is small (<5 cm and 3 mm in length and diameter respectively); it emerges on the ground surface layer or underneath dung pats that have usually experienced more than 25 days of decomposition; an independent field investigation showed that it primarily feeds on cattle dung and its density may reach 80 individuals per pat. The latter species is larger (>10 cm and 0.5 cm in length and diameter respectively), and it can be found scattered in mesophytic habitats with a variable population density (c. 5–50 individuals m−2) in our study site; but it often reaches high densities near dung pats. We observed that the large earthworms are often active at the surface soil layer (<0–10 cm), principally feeding on plant litter detritus and soil organic aggregates, and rarely appear above the soil surface unless the soil is saturated with water. We occasionally observed above-ground predators (e.g. birds and frogs)
preying on the worms, but only when they were out of the cryptic habitats (underneath dung pats and in soils for the small and large species respectively). However, a species of predatory beetle, *Agonum impressum*, may commonly prey on the earthworms. This beetle is about 8 mm long on average and it is solitary, with densities ranging between 0-6 and 2-2 individuals m$^{-2}$ (based on an independent survey of 20 1 x 1 m quadrats). The beetles may forage into dry dung pats and go down into the soil through small openings. We have observed this beetle dragging dead bodies of the large and small earthworm species out of the soils and cutting them into pieces to eat. This directed us to perform a pre-experiment, in which a beetle and worms of each species were put into the same chamber (diameter = 25 cm, height = 50 cm) filled with soil (10 cm in depth). An adult beetle completely consumed two of the smaller worms within 1 day, and two of the larger worms within 1 week. On the basis of overlapping habitat use, field observations and our predation trial, we anticipated that the beetle species is a potential predator preying on the earthworms under natural conditions.

Abundant literature has suggested that earthworms are able to change soil physical and chemical conditions, mediate decomposition of organic material and enhance plant growth (e.g. Edwards 2004). Moreover, both the earthworms and beetles are active during the growing season, which extends from late June to late August at our study site. We therefore focused on the relationships among the beetle, the worms, soil properties, dung decomposition and plant growth in the alpine meadow.

**EXPERIMENTAL DESIGN**

The experiment was conducted for 4 months, from June through September, 2011, in a fenced 0.5 ha plot in our study site. The experiment included three treatments, all containing soil, plants and a dung pat: (i) control (neither earthworms nor predatory beetles added); (ii) predator-absent (earthworm added only); and (iii) predator-present (beetles and earthworms added) (see Fig. S1 in Supporting Information). Each treatment was replicated 10 times; all the replicates were randomly deployed at the field site (at a minimum spacing of 2 m) after the following described set-up. For each replicate, a circular core (30 cm x 20 cm, diameter x depth) of above- and below-ground plant materials and soil was first trenched and then carefully extracted from the ground. Because plant roots are usually <20 cm in depth and are very dense in the meadow, we were able to remove the whole plant-root-soil system intact during coring. Subsequently, cores were transplanted into cylindrical chambers (30 cm x 30 cm, diameter x depth), with sides made of 0.5 mm aluminium sheeting, and a bottom made of a steel screen (0.3 mm thick). The mesh size of the screen was 0.5 x 0.5 mm, which prevented small earthworms from escaping and allowed water to drain. Then, the electro-shocking method was applied to remove pre-existing earthworms from each chamber using self-made stainless steel probes (6-4 mm in diameter and 30 cm in length) and a portable generator (to generate an electric current ~2 Amps), following Fonte et al. (2007). After adding water into the chamber to bring the soil near field capacity, we inserted four evenly spaced probes into soil, removing worms for a total of 15 min, after which no more worms were found. Then, these experimental chambers were placed back in the ground. In addition, the chamber was further fitted with an above-ground cylinder net, which was made of the same screen as the chamber bottom. The tops of chambers were covered with screen to prevent beetles or earthworms from entering or leaving (Fig. S2). Measurements made on sunny days showed that the light intensity under such screens was about 80% of full sunlight.

Before starting the experiment, fresh yak dung was collected in the early morning from a stall of a Tibet family. The dung was thoroughly mixed, and then divided into individual pats using a circular metallic mould before addition of a single pat into each experimental chamber. The pats were about 15 cm in diameter and 1000 ± 50 g in fresh weight (± 4 cm thick). The small earthworms were hand-sorted from beneath dry dung pats, both the large worms and beetles were collected from surface soil by hand. These collections were completed within 2 days, until enough individuals were captured to conduct the experiment. We selected medium-sized adults of each species (10-4 cm and 3.5 cm in length on average for the large and small species respectively) for the experiment. Two beetles (one male and one female) were added to each replicate of the predator-present treatment; 4 large worms and 30 small worms were added to each replicate of predator-present and predator-absent treatments. The experimental density of worms was at the upper range of that observed at our field site and the relatively low predator to prey ratio was established to allow interactions among beetles, worms, soil and plants to materialize over the 4 month growing season.

**RESPONSE VARIABLES**

At the end of the experiment, the remaining dung was collected and the above-ground plant parts (except those that senesced under the original dung pats) were harvested from each chamber. To facilitate examination of possible earthworm behavioural responses to predation risk and corresponding impacts on soil physical and chemical properties, we separately quantified all below-ground response variables within both the upper (0-10 cm depth) and the lower soil layers (10-20 cm depth). Three soil cores (5 cm in diameter and 20 cm in depth) beneath the harvested plants (not under dung pats) were sampled from a sub-set of each treatment (N = 5; not fully sampled due to limits of time and labour) for the measurement of soil bulk density (at both upper and lower soil layers, see below). Then we separated the remaining soil left in each chamber from the steel cylinder in one large piece and placed it on a plastic sheet, where it was quickly cut into upper and lower halves as detailed above. For each layer, we manually sieved the soil, and picked out and counted all plant roots, worms (including the living and dead mature ones, eggs and juveniles, if any) and beetles (including the living and dead, if any). Subsequently, the soil was mixed, and 500 g samples were collected. In addition, soil cores that were initially utilized for bulk density measurements were separated into upper and lower soil layers, and worms and beetles (if any) were subsequently sorted and counted, and the soil placed in containers. We did not observe other soil macro-invertebrates (> 2 mm in size) during the investigation, and we did not examine the soil meso- or micro-invertebrates < 2 mm at the termination of the experiment, although they could be abundant (Xiao et al. 2012). All experimental chambers were harvested and processed within 24 hrs.

The above-ground parts of plants were dried at 75 °C for 48 hrs and separated into grass and forb functional groups, and weighed. The soil samples, together with the containers (that included the soil for bulk density measurements), were weighed immediately to obtain fresh soil weight for each soil layer, dried at 105 °C for
48 hours and weighed for the calculation of soil water content. Soil bulk density was calculated as the dry mass of soil in the container divided by half the volume of the soil core. Moreover, for each soil sample total N and P were determined by the Kjeldahl method and spectrophotometric Colorimetry (Unicam-200, Unicam, Cambridge, UK), respectively; available N and P concentrations were determined using the alkaline KMnO4 method and 0.5 M NaHCO3 (pH 8.5) solutions respectively.

As the vertical distribution of the earthworms could only be determined when the cores were destructively sampled, we were only able to measure this response on a single occasion (at experiment termination) in the experiment. Therefore, in parallel with the above experiment (hereafter called the ‘primary experiment’), we conducted a second (‘accessory experiment’), to examine how the vertical distribution of the earthworms likely varied during the course of the primary experiment and thus aid interpretation of the of vertical behavioural response of the earthworms. The design of the accessory experiment was identical to that of the primary experiment (3 treatments, N = 10), but the cores were destructively sampled on the 32nd and 55th days after initiation of the experiment (N = 5 each time point). The vertical distributions of the earthworms were examined using the same method as for the primary experiment.

**DATA ANALYSES**

To determine effects of predators on the dry mass of residual dung, multiple soil properties and plant biomass, we used a one-way ANOVA for each respective response variable. In these analyses, levels of the fixed treatment factor consisted of the control, predator-absent and predator-present treatments. Post-hoc Tukey tests were used to further elucidate treatment differences. We used a Student’s t-test to compare earthworm density across predator-absent and predator-present treatments; i.e. test for a consumptive effect of predators. We constructed a generalized linear mixed model (GLMM, with binomial distribution) to determine the difference in the proportion of earthworms in the upper soil layer (i.e. responsible variable) across predator-absent and predator-present treatments and thereby assess non-consumptive, vertical habitat shifting, effect of predators. We first ran the model for the pooled data set including both the primary and two accessory experiments, with the treatment (predator present vs. absent) and earthworm species being fixed factors and the experiment type a random factor, and then ran the model without the random factor followed by Tukey tests for each of the experiments. Consumptive and non-consumptive effects were assessed for small and large worms separately. In addition, to further determine if there were significant associations among variables concerning soil feature and plant growth, regression analysis was used to examine the effects of soil bulk density on water content, of water content on N availability and of N availability on above-ground plant biomass. Where parametric tests were performed, data were first checked for normality and variance homogeneity. The data on earthworm density were log-transformed before analysis to meet these assumptions. All the data analyses were conducted in R (R Development Core Team 2011).

**Results**

In the predator-present treatment, an average number of 1.72 ± 0.11 beetles (mean ± 1SE, N = 20), were recovered at the end of the experiment - producing a mean predator survival rate of 0.86. None of the beetles were found in the lower layer of soil. There were no active juveniles found for either worm species in the two treatments in which worms were stocked. The total number of the earthworms recovered at the end of the primary experiment was comparable between the predator-present and absent treatments for both species (average 3.66 and 3.63 large worms and 20.5 and 19.2 small worms for the predator-absent and present treatment, respectively; N = 20, large: t = 0.06, P = 0.528; small: t = 0.956, P = 0.827). Therefore, we did not detect any predator-driven suppression of overall earthworm densities. However, we detected a predator-induced vertical habitat shift of earthworms. The presence of predators shifted the vertical distribution of large earthworms from the upper to the lower soil layer; in the absence of predators, an average of 80% of large worms were found in the upper soil layer, compared with only 35% in the presence of predators (Fig. 1; see report of GLMM in Table S1 in Supporting Information). For the small worm species, the effect of predator presence on vertical distribution was statistically non-significant (Table S2). In the accessory experiment, large worms were much more abundant in lower layer than in upper layer in the predator-present treatment than in the predator-absent treatment for both sampling times, but the difference was non-significant for the small species (Fig. S3). The results of GLMM of the pooled data set further showed that small worms were more likely to remain at upper soil layer, such that their responses to predators were less apparent than large worms (Table S1). The behavioural responses in the accessory experiment thus supported those observed in the primary experiment and provided evidence that these effects were temporally consistent during the experiment.

![Fig. 1. The effects of predatory beetles on the vertical distribution of the earthworm species (as shown by proportion of earthworms in the upper layer) in the predator-absent (grey column) and predator-present treatments (black column). The different letters above the error bars denote differences between treatments and between layers were statistically significant at the level of P = 0.05, as revealed by a generalized linear mixed model with binomial distribution. Error bars are SE.](image-url)
In the absence of predators, earthworms did not affect soil bulk density in either upper or lower layers (relative to the control), but in the presence of predators, earthworms significantly reduced soil bulk density in the lower layer and, notably, not in the upper layer (Fig. 2a; see report of ANOVAs in Table S2 in Supporting Information). In addition, soil water content in the lower layer was higher in the presence of predators relative to the control and predator-absent treatments, and there were no significant differences in the upper layer (Fig. 2b; Table S2).

In the upper soil layer, soil organic matter content, total N and P were all significantly greater in the predator-absent treatment than the control and the predator-present treatment. Conversely, in the lower soil layer, soil organic matter content, total N and P were highest in the the predator-present treatment (Figs. 2c, d, e; Table S2). Similar trends were also observed for available N and P (Fig. 2f, g; Table S2). The dry mass of residual dung on the ground surface was indistinguishable among the treatments (Fig. S4).

**Fig. 2.** The difference in soil properties including (a) soil bulk density, (b) water content, (c) organic matter content, (d) total nitrogen, (e) phosphorus content, (f) available nitrogen and (g) phosphorus among the control (white column), predator-absent (grey column) and predator-present treatments (black column) for both lower and upper soil layers. The different letters above the error bars denote the difference among treatments (but not between layers) was statistically significant at the level of $P = 0.05$, as revealed by one-way ANOVA followed by Tukey’s test for the upper and lower soil layers respectively. Error bars are SE.

Above-ground plant biomass was indistinguishable between the control and the predator-absent (worm only) treatments ($P = 0.164$, see Table S2). However, above-ground plant biomass was much higher (and statistically significantly so) in the predator-present (worm and beetle) treatment, which exceeded both the control (by 37.6%) and predator-absent (by 20.2%) conditions (Fig. 3). Similar trends held for plant biomass of both grass and forb species (Fig. S5). No significant difference was found for root biomass among the treatments. Notably, however, there was a trend towards higher values in the predator-present treatment at both lower and upper soil layers (Fig. S6).

In the lower soil layer, there was a negative linear relationship between soil bulk density and water content (Fig. 4a), a positive linear relationship between water content and N availability (Fig. 4b), and a strong positive linear relationship between N availability and total above-ground biomass across all of our experimental treatments (Fig. 4c). These linked soil density - soil nutrient - plant biomass relationships were not evident within the upper soil layer.

**Discussion**

Contrary to the ‘brown-world’ hypothesis, we found that predators actually indirectly facilitate plants via the detritus food web in our alpine meadow study system. Our results indicate that this can be attributed to a TMII driven by the vertical habitat-shifting behaviour of earthworms that led in turn, to a strengthening of their positive effect on plant growth.

We predicted that in the absence of predatory beetles earthworms would improve soil conditions and enhance plant biomass. Our results support the first, but not the second, of these predictions. Earthworms alone enhanced soil conditions, as evidenced by higher nutrient concentrations in both upper and lower soil layers in the predator-absent (worm only) treatment relative to the control. Earthworms likely increased release of soluble nutrients through decomposition of plant litter and soil organic particles. Furthermore, their burrowing and subterranean movement likely facilitated soil aeration and root respiration, which could be especially important for plant growth and microbial growth in densely rooted alpine meadow systems (Cao et al. 2004). However, these improvements in soil conditions were not translated into a marked enhancement of above-ground plant biomass in the earthworm-only treatment relative to the control during our experiment. The absence of an effect can be tentatively attributed to (i) the fact that soil of the upper layer was in good condition at the start of the experiment and remained so in the absence of worms (see values for controls); and (ii) the duration of the experiment was not long enough to differentiate weak effects of worms on plant biomass accumulation. However, the general tendency of earthworms to increase plant growth was nevertheless suggested by our data (although the strength is weaker than in other studies; e.g. Edwards 2004; Laossi et al. 2010) that indicates plant biomass increased by

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**Fig. 3.** The difference in above-ground plant biomass among the three treatments. The different letters above the error bars denote the difference was statistically significant at the level of $P = 0.05$, as revealed by one-way ANOVAs followed by Tukey’s test. Error bars are SE.

**Fig. 4.** The regression relationships showing the effects of (a) soil bulk density on water content ($N = 15$), (b) of water content on N availability ($N = 30$), and (c) of N availability on above-ground plant biomass ($N = 30$). The decision coefficient ($R^2$) and $P$ values are provided.
nearly 15% - a substantial increase over a short experimental duration in highly productive meadow (Li et al. 2011). Importantly, this increase in plant biomass is consistent with the variation pattern observed for soil nutrient conditions. Our results are thus not inconsistent with the widely found plant growth-promoting role of earthworms in terrestrial plant-based natural and agricultural systems (e.g. Dominguez, Bohlen & Parmelee 2004; Edwards 2004).

In contrast to our prediction that predatory beetles would reduce plant growth, our results revealed a positive effect of predatory beetles on plant biomass accumulation. This counterintuitive finding can be partially explained by the fact that earthworms alone had only a weakly positive (and statistically non-significant, see above) effect on plant biomass, thus limiting the potential for a negative DMII driven by predatory beetles in our system. Moreover, the addition of the predatory beetles did not significantly decrease the density of either earthworm species, further precluding a DMII. The non-existence of a predator-mediated reduction in earthworm density was presumably because the beetles fed on the alternative soil food resources such as springtails and mites (Xiao et al. 2012), while worms were either non-preferred, or able to effectively escape predation risk. Nevertheless, given the positive effect of earthworms on soil properties, predatory beetles would not be expected to enhance plant biomass, as we found. The explanation for the emergence of a positive indirect effect of beetles on plants lies in predator-induced behavioural changes of earthworms, i.e. a vertical habitat shift of earthworms from upper to lower soil levels.

Our results show that a high proportion of large earthworms moved down into the lower soil layer in the presence of predators, presumably to reduce direct contact with the beetles and associated risk of predation following perception of predator vibrations and odours (sensu Cataonia 2008), while small worms showed a much weaker and non-significant response. It is beyond the scope of this study to elucidate the underlying cause of this difference, but we can speculate that it could be due to one or more of the following factors: (i) greater energetic cost of the larger distance (relative to body size) for the smaller worms to relocate to the lower soil layer; (ii) burrowing strength limitations of smaller worms preventing penetration of compacted lower soil layer; or (iii) asymmetrical competition between large and small worms that reduced the net benefits of small worm movements. Note that isolation of interactions between the large and small earthworms was not necessary to test our hypothesis and thus we did not explicitly test for this interaction in our analyses. Nevertheless, both worms shifted in the same direction and this predator-induced vertical habitat shift changed conditions in the lower soil profile resulting, in turn, in a positive effect of predatory beetles on plants. It is important to note that in our study system, the lower layer of the soil is generally barren in terms of available nutrient and water content, and also has low organic matter content (see values for controls). Indeed, soil profiles of grassland systems often exhibit declining nutrient concentrations, porosity and water content with increasing depth (Doudill, Heathwaite & Thomas 1998; Marschner & Rengel 2007). The condition-improving activity of the earthworms in the otherwise highly unfavourable lower soil layer may therefore have stronger effects on plant growth than similar activities in the upper soil layer. Although an increase in local density per se (i.e. aggregation) could also conceivably generate TMII (sensu McIntire & Hik 2005; Olofsson 2006; Gao et al. 2007), this can largely be ruled out as an explanation of the TMII in our study because densities of large worms were comparably high (and even slightly higher) in the upper soil layer in the absence of predators as compared with the lower soil layer in the presence of predators, pointing to a shift in the vertical distribution of worms as the primary explanation of the observed TMII.

Our results show that the shifting distribution of earthworms to the lower soil layer in the presence of predators increased soil porosity (lowering bulk density), in turn facilitating water drainage and nutrient leaching from the upper soil layer (see also Dominguez, Bohlen & Parmelee 2004). Moreover, the dry mass of dung was higher in the controls than in both of the earthworm-containing treatments (even though there was no difference between the two earthworm-containing treatments, i.e. predators present and absent treatments), suggesting that during the vertical movement of the earthworms, soil organic particles and the cattle dung might have been transported from the ground surface into the soil. In the predator-present treatment, this organic matter, if transported to the deep soil, may be more likely to release soluble nutrients into the lower soil layer due to earthworm digestion, possibly together with improved microbial activity (due to improved soil aeration). These improvements in plant growing conditions were clearly reflected in increased above-ground plant biomass and a simultaneous, albeit non-significant, increase in root biomass. The mechanistic links between earthworm-modified conditions in the deep soil layer and plant biomass were further evident in the continuous linear relationships among soil bulk density, water content, N availability and above-ground biomass in this zone.

It must be noted that several other mechanisms related to predator presence may have also contributed to the observed positive effects of predators on plant biomass. First, conceivably the addition of predators may have relaxed inhibition of plant growth caused by a concentration of earthworms in the upper soil layer, as suggested by studies showing high density of earthworms may negatively affect plant growth in some specific conditions (Fonte et al. 2007; Laossi et al. 2010). This mechanism is not, however, supported by our data that show a slight positive effect of earthworms (in the absence of predators) on plant biomass. Second, the beetles may have consumed
plant antagonists (e.g. tipulid or elaterid larvae; Xiao et al. 2012) in the experiment and hence indirectly facilitated plant growth through an alternative pathway than the one proposed here; or beetles may have facilitated the growth of microorganisms beneficial to plants (e.g. arbuscular mycorrhizal fungus) by changing the species composition and structure of the soil micro-fauna community. Although we cannot rule out these latter two mechanisms contributed to the observed effect of beetles on plant biomass, the strong linkages between predator presence, earthworm vertical distribution and soil properties in the lower soil zone and plant biomass shown in our results suggest that the trait-mediated indirect interaction chain we discovered is the primary mechanism driving the observed increase in plant biomass in the presence of predators.

Our findings contrast those from predator–herbivore–plant interaction chains, in which TMIIIs (measured across the whole plant level) follow predictions of classic theory and are almost invariably positive, strengthening DMIIIs (reviewed by Schmitz, Krivan & Ovadia 2004). In the case of both herbivores and detritivores, the predator-induced trait shifts are theoretically driven by a trade-off between energy gain and predation risk (Downes 2001). The energy gained by herbivores from their plant resources tends to be tightly related to the negative impact they have on the standing stock of their resources (plants) (e.g. Schmitz 2008). However, the energy gained by detritivores from their resources may be less tightly coupled to their positive effects on plants because these effects also depend on non-trophic mechanisms (e.g. translocation of nutrients or ecosystem engineering activities) that may remain unaffected or may even be enhanced by predator presence. Therefore, in predator–detritivore–plant systems, we expect TMIIIs to be more variable relative to DMIIIs than in predator–herbivore–plant systems. Moreover, such TMIIIs may even generate net effects counter to those predicted by classic trophic cascade theory, as reported here.

The vertical habitat shift of earthworms in response to predator presence that we propose drove the positive TMII can be explained in the light of their habitat domain relative to that of their predators (sensu Schmitz, Krivan & Ovadia 2004). The earthworms may move both horizontally and vertically in the soil column to forage and feed, and in a field survey we observed that the large ones are active at the depth of 30 cm. However, the beetles mostly move on the ground surface and appear to rarely go down into soil foraging. Thus, the domains of the beetles and the worms are oriented perpendicularly, potentially allowing the worms to escape from risk of predation by the beetles by moving vertically as opposed to horizontally. This contrasts to the relative habitat domains of predatory beetles and a detritivorous beetle studied by Wu et al. (2011), both of which were primarily ground-dwelling with overlapping horizontal domains, precluding a spatial refuge for the detritivore. Indeed, concordant with our argument above (see also Schmitz, Krivan & Ovadia 2004), Wu et al. (2011) reported strong predator suppression of detritivore density with no evidence of a detritivore habitat shift or other form of TMII. Although not universal, we forward that predator-induced vertical habitat shifts of detritivores to deeper soil layers will occur where above-ground predators (e.g. predatory beetles, frogs and birds) and burrowing detritivores (e.g. earthworms and beetles; Lima & Dill 1990; Dyer & Letourneau 2003; Croll et al. 2005; Wardle 2006) have perpendicular habitat domains - a combination which may be widespread. We further postulate that, if per capita effects of detritivores are found to be generally greater in deeper soils (as in this study), the emergence of a positive TMII between predator and plants via detritivores may be a predictable general consequence of such vertical habitat shifts. We note, however, that assessing the generality of our findings await further empirical tests and may be complicated by factors such as interspecific variability in plant rooting depth and variation in soil profiles across sites and systems.

The recent ‘brown-world’ hypothesis (Allison 2006; Wu et al. 2011) forwards a long-overlooked negative indirect effect of predators mediated through direct trophic interactions with detritivores. In stark contrast to this prediction and the study result of Wu et al. (2011), that was conducted on the same alpine meadow study sites and revealed a strong DMII between predatory beetles and dung-dwelling coprophagous beetles, we found that the predators of earthworms actually promote plant growth principally by driving key soil-modifying detritivores to deeper soil layers. This shows that predator-induced habitat shifts of detritivores may reverse the sign of indirect interactions between predators and plants that would be predicted by logical extension of classical trophic cascade theory. Further studies in a range of systems are required to assess the potential generality of these results. Nevertheless, our findings suggest that TMIIIs will have to be explicitly included into below-ground interaction webs if the effects of predators on detritivores, soil properties and associated above-ground ecosystem functions are to be successfully predicted.

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References


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**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** A diagram showing the three experimental treatments and graphical representation of results of the experimental manipulations. The top three panels show the treatments at initiation of the experiment and the lower three those at the end of the experiment. The difference in plant size denotes the above-ground biomass difference among treatments; the earthworm distribution at different soil layers varied between the earthworm and beetle treatments. See text for more details.

**Fig. S2.** A graphic representation of the physical setting of this study. The total fresh weight of soil, dung pat and plants was 21 ± 1.2 kg (N = 10). See text for more details.

**Fig. S3.** The effects on predator beetles on the vertical distribution of the large and small earthworm species in the predator-absent (grey column) and predator-present treatments (black column) for...
the first (a) and second (b) sampling in the accessory experiment. The different letters above the error bars denote the difference between treatments and between layers was statistically significant at the level of $P = 0.05$, as revealed by a generalized linear mixed model with Poisson errors.

**Fig. S4.** Variation among the three treatments in the dry mass of residual dung on the soil surface at the end of the experiment. There is no statistical difference among the three treatments at the level of $P = 0.05$, as revealed by one-way ANOVA.

**Fig. S5.** Variation in plant biomass among the three treatments of (a) forb and (b) grass species at the end of the experiment. The same letters above the error bars denote the difference was statistically insignificant at the level of $P = 0.05$, as revealed by one-way ANOVA followed by Tukey’s test.

**Fig. S6.** Variation among the three treatments in root biomass at the end of the experiment, for both the lower and upper soil layers. The same letters above the error bars denote the difference between treatments (but not between soil layers) was statistically insignificant at the level of $P = 0.05$, as revealed by one-way ANOVAs followed by Turkey’s test for the upper and lower soil layer respectively.

**Table S1.** Results of generalized linear mixed model (with binomial distribution) showing the effects of predatory beetles, earthworm species and their interactions on proportion of earthworm at upper soil layer for the primary experiment and the accessory experiment, and the pooled data set.

**Table S2.** Results of one-way ANOVA showing the treatment effect on plant biomass, dry mass of residual dung, proportion of large and small earthworms at upper layer, soil organic matter, total N and P, available N and P, water content and soil bulk density at both upper and lower layers. Tukey tests were used to determine the difference among three treatments (if any) following the ANOVAs.