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論文名 植物の生残と成長におよぼす地下部の根食と
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Experimental studies on ecological effects of belowground herbivory
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環境不均質性の影響の実験生態学的研究（英文）

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Summary

Plant growth and survival depend on environmental heterogeneity and soil organisms. In soil environments, water and nutrients that are essential for plants are heterogeneously distributed at various scales. Plant roots and soil fauna respond to the heterogeneous distribution of resources in space and time, and distribution of roots and soil fauna, consequently, changes. Thus, soil resource-heterogeneity, fauna, and their interaction could be attributed to the plants fate.

Herbivory alters plant growth, survival and the mutual interactions of plants. Plant damage from herbivory in belowground environments (belowground herbivory) can be more severe compared with those caused in the aboveground environment because belowground herbivorous insects (belowground herbivores) sever primary roots, detaching most parts of the root system. The effects of belowground herbivory on plants depend on soil water and nutrients because these two factors alter the dynamics of plant roots and belowground herbivores. In general, belowground herbivory is considered to have more severe impacts on plant performance under low soil water and nutrient availability. However, few studies have reported the effects of soil water and nutrients on plants through changes in belowground herbivores. Moreover, the effects of the heterogeneity of water and nutrients on belowground plant-herbivore interactions have been rarely examined.

In this thesis, I conducted four pot experiments to elucidate the interactions between belowground herbivory and soil heterogeneity. I used two plant species, *Lolium*

perenne and *Plantago lanceolata*, which co-occur in seminatural temperate grasslands, and generalist root-chewing insects (Scarabaeidae: Coleoptera), the most dominant group of generalist root chewers.

I investigated the effects of the belowground vertical distribution of a herbivore on plant biomass and survival in *L. perenne*. One *L. perenne* plant was grown in a pot with a larva of *Anomala cuprea*. The larva was confined in the pot to different vertical distributions: top feeding zone (top treatment); middle feeding zone (middle treatment), or bottom feeding zone (bottom treatment). Alternatively, pots contained no larva (negative control), or larval movement was unconfined (positive control). In the top treatment, seven of the nine plants died and plant biomass was significantly lower compared with the negative control. In the bottom treatment, no plants died and plant biomass showed no significant differences. In the positive control, seven of the nine plants died. Most belowground herbivores congregate near the soil surface, severing the roots from the shoot.

I investigated the effects of belowground herbivory on the survival and biomass of *L. perenne* and *P. lanceolata* at various growth stages. *L. perenne* or *P. lanceolata* plants at four different growth stages were grown in pots with or without a larva of *A. cuprea*. Herbivores caused significant plant mortality at the early growth stages in both species but no plants died at the last growth stage. High mortality and most biomass loss in *P. lanceolata* at the early growth stages were attributed to herbivory, but mortality and biomass loss were negligible at the last stage. In contrast, herbivory reduced the biomass of *L. perenne* at all stages. These results show that the effects of

belowground herbivory changed with differing growth stages and the root architecture of plants.

I found that water availability and temporal heterogeneity affected the vertical distribution and mortality of a belowground herbivore, and consequently plant growth. *P. lanceolata* seedlings were grown (one per pot) under different combinations of water volume (large or small) and heterogeneity (homogeneous water conditions, watered daily; heterogeneous conditions, watered every four days) in the presence or absence of a *A. cuprea* larva. The larva was confined in different vertical distributions to top feeding zone (top treatment), middle feeding zone (middle treatment), or bottom feeding zone (bottom treatment). Alternatively, no larva was added (control) or larval movement was not confined (free treatment). With a small water volume treatment, the plant biomass in the free treatment decreased with the homogeneous water supply, but increased in the heterogeneous water supply. Plant biomass in the free treatment under the homogeneous water supply was similar to the top treatment and under the heterogeneous water supply was similar to the middle and bottom treatments. The heterogeneous supply of the small-volume water treatment caused the herbivores to move deep into the soil profile, avoiding drought conditions at the soil surface. With a large water volume treatment, the plant biomass under the homogeneous and heterogeneous water supply declined in the free treatment. Plant biomass in the free treatment was similar to that of the top treatment regardless of heterogeneity. These results suggest that herbivore distribution occurred in shallow soil, reducing root biomass. Herbivore mortality was high with a heterogeneous

supply of small water volumes or a homogeneous supply of a large water volume. With a large water volume, plant biomass was high with significant herbivore mortality.

I investigated the interactive effects of soil nutrient heterogeneity and belowground herbivory on the growth of plants with different root-foraging traits. One *L. perenne* seedling, a precise root-foraging species, and a seedling of *P. lanceolata* were grown in pots under different patterns of nutrient distribution (homogeneous or heterogeneous) with or without a belowground herbivore (a *Popillia japonica* larva). With no herbivore, the biomass of *P. lanceolata* was smaller in the heterogeneous nutrient treatment compared with the homogeneous treatment, and the biomass of *L. perenne* was not different between homogeneous and heterogeneous treatments. Under homogeneous nutrient distribution, the biomass of both plant species was smaller with the herbivore. Under the heterogeneous nutrient treatment, plant biomass was reduced by the herbivore only in *L. perenne*. The roots of the precise root-foraging species were grazed more under the heterogeneous nutrient distribution.

Plant damage depends on the amount of root loss that was different with the vertical distribution of the herbivore, plant growth stage and root architecture. If belowground herbivores occurred in shallow soil at the early growth stage of plants, many plants lost most of their root biomass and died. The belowground herbivores are generally distributed in shallow soil in the fields, thus belowground herbivory would prevent seedlings of a specific species from establishing and reduce its abundance because the species are vulnerable to belowground herbivory.

Heterogeneity of water and nutrients in the soil altered the plants and belowground herbivores, affecting belowground herbivory on the plants. The burrowing of *A. cuprea* larva deeper into the soil profile was attributed to the dry soil surface conditions from the small and heterogeneous supply of water, causing a negligible effect on the plants. Belowground herbivory is expected to damage plants under low soil water availability. However, this effect may not always occur because belowground herbivores could change their foraging behavior in response to heterogeneous soil moisture, and nutrient heterogeneity in soil would alter root abundance and distribution, and the effects of herbivory on plants. Thus, the heterogeneity of soil water and nutrients should be considered in any future studies of belowground plant-herbivore interactions.

Chapter I

General Introduction

Plant growth and survival depends on environmental heterogeneity and soil organisms. In soil environments, essential resources for plants such as water and nutrients are heterogeneously distributed at various scales. Plant roots respond to the heterogeneous distribution of resources in space and time, and distribution of roots, consequently, changes. Soil contains a variety and abundant level of organisms that can affect plant performance. Thus, resource heterogeneity and fauna in soil could be attributed to plant fates, which has been rarely studied using empirical and theoretical methods.

Belowground herbivores that feed on living plants are underestimated in ecological studies because soil ecosystems have been considered as decompose systems in which soil organisms break down detritus. However, belowground herbivores that are abundant in soil directly graze on live roots and their effects on plants could be crucial. Therefore, belowground herbivores and the heterogeneity of soil resources that indirectly determine root abundance and distribution, may interactively determine plant growth and survival.

In this chapter, I review research on belowground herbivory and the interactive effects of soil resources and belowground herbivory on plants. I then propose hypotheses that are investigated in this thesis.

Belowground herbivores and their effects on plants

I review the research on belowground herbivorous insects and their effects on plant growth and survival. In addition, I highlight the dominant organisms and features of belowground herbivory. Belowground plant-herbivore interactions are less frequently studied compared with aboveground interactions (Andersen 1987; Brown and Gange 1990; Hunter 2001) despite their substantial impacts on plant survival (e.g., Strong et al. 1995; Coverdale et al. 2012). The relatively small number of ecological studies on belowground herbivory is attributed to experimental difficulties such as belowground herbivores not being highly visible (Brown and Gange 1990; Hunter 2001; van Dam 2009). Therefore, this may influence the type of research carried out by ecologists.

In the last two decades, ecologists have growing interest in belowground interactions between plants and herbivores, because the herbivores influence plant community dynamics (Brown and Gange 1992; Gange and Brown 2002; De Deyn et al. 2003; van der Putten 2003; Schädler et al. 2004; Wardle et al. 2004; van der Putten et al. 2009; Stein et al. 2010) and aboveground multitrophic interactions (Poveda et al. 2007; Johnson et al. 2008; van Dam and Heil 2011; Soler et al. 2012). However, most of the studies give little attention to individual traits of a plant or a belowground herbivore, and to environmental conditions affecting these traits. This may explain the lack of perspective on belowground interactions between plants and herbivores. Therefore, more detailed studies are required on these types of interactions.

Dominant belowground herbivores in fields: generalist root-chewing insects

Insects are important belowground herbivores, occurring in the soil and feeding on subterranean plant parts (Andersen 1987; Brown and Gange 1990; Mortimer et al. 1999; Whittaker 2003). The root-chewing insects belonging to the order Coleoptera and Diptera, are the most abundant in soils (Mortimer et al. 1999). More than 75% of belowground herbivores found in a seminatural grassland in the UK, belonged to the Scarabaeidae and Elateridae (Coleoptera) and Tipulidae (Diptera) (Brown and Gange 1992). All the belowground herbivores in the subalpine grassland were from the orders Coleoptera and Diptera (Morón-Ríos et al. 1997).

Generalist root-chewing insects are relatively well studied as they are dominant in fields. Moreover, plant damage by root chewers are more severe compared with sap feeders or root borers because root chewers reduce the most root biomass and the generalists are more detrimental to plant fitness compared with specialists regardless of feeding type (Zvereva and Kozlov 2012). Therefore, I used generalist root-chewers from Scarabaeidae (Fig. 1-1), the most dominant group of generalist root chewers (Whittaker 2003; Zvereva and Kozlov 2012) in grasslands (Seastedt and Murray 2008).

The effects of belowground herbivory on plants

Belowground herbivores especially root chewers, ruin larger amounts of roots than the

ingested amounts because the herbivores sever the primary roots and detach most parts of the root system (Murray and Clements 1992, 1998; Staley and Johnson 2008; Erb and Lu 2013). Therefore, belowground herbivory cause substantial biomass loss because most plant biomass occurs belowground rather than aboveground. Belowground herbivory may affect the plant more significantly compared with folivory, because belowground biomass is estimated to consist of over 80% of plant biomass in temperate grasslands, tundra and deserts (Jackson et al. 1996; Mokany et al. 2006).

Belowground herbivory significantly damages belowground biomass but indirectly affect aboveground biomass. There are several detrimental effects on aboveground biomass (Zvereva and Kozlov 2012) such as: (1) decreased water and nutrition uptake from root biomass loss; (2) decreased photosynthetic rates from the water deficit caused by root damage; (3) decreased assimilate allocation to aboveground plant parts enhancing root regrowth; (4) loss of stored resources; and (5) the cost of induced defenses. These negative effects are attributed to the reduction in the absorption of belowground resources such as water and nutrients.

The negative and positive effects of belowground herbivory on plants

The effects of belowground herbivory on plant growth and survival are not unidirectional (Seastedt and Murray 2008; Erb and Lu 2013). Müller-Schärer (1991) found that belowground herbivory diminished the biomass of *Centaurea maculosa*, but enhanced

competitive abilities because grazed roots exuded allelopathic chemicals (Thelen et al. 2005). The example of *C. maculosa* demonstrates that belowground herbivory has a negative and positive effect on plants under competition.

Species dependent effects of belowground herbivory on plants

The damage from belowground herbivory on plant performance varies depending on the plant species (e.g., Sonnemann et al. 2012). Belowground herbivory affects grasses more than forbs at mature growth stages (Zvereva and Kozlov 2012). These different herbivory effects result in morphological differences between grasses and forbs, especially in the roots (Zvereva and Kozlov 2012). Belowground herbivory at a similar magnitude may have different effects on a plant according to morphological traits because each plant species has its own morphology (Mortimer et al. 1999). This scenario is applicable to other plant traits, which may alter the effects of belowground herbivory.

The effect of belowground herbivory on plant performance varies depending on the species of the belowground herbivore (e.g., Steinger and Müller-Schärer 1992; Wurst and van der Putten 2007) and density (e.g., Masters 1995; Onstad et al. 2006). For example, larvae of the red milkweed beetle, *Tetraopes tetraophthalmus*, feeding on the taproot of *Asclepias syriaca* reduce root biomass, but the click beetle larvae (mainly *Hypnoides abbreviatus*) grazing on *A. syriaca* fine roots do not reduce root biomass (Erwin et al. 2013). The biomass of *Lantana camara* showed no significant change under

low densities of the root-feeding flea beetle, *Longitarsus bethae*, compared with the no-beetle control, but root biomass decreased under high densities of the beetle (Simelane 2010).

The occurrence of natural enemies on some belowground herbivores affects belowground plant-herbivore interactions. For example, the entomopathogenic nematode, *Heterorhabditis marelatus*, parasitic on ghost moths, prevented lupine dieback (Preisser and Strong 2004).

The effects of soil water and nutrients on belowground herbivory of plants

All organisms in the soil community, including plants and herbivores depend on abiotic soil factors (Bardgett 2005; Barnett and Johnson 2013; Erb and Lu 2013). Water and nutrients are the most important abiotic factors in soils, because water primarily determines plant productivity in many terrestrial biomes (Webb et al. 1986; Churkina and Running 1998; Heisler-White et al. 2008) and is a dissolvent allowing plants to absorb soil nutrients.

Belowground herbivory reduces plant root biomass, causing a resource deficit in plants (Blossey and Hunt-Joshi 2003; Zvereva and Kozlov 2012; but see Crutchfield et al. 1995). Therefore, belowground herbivory is considered to have more severe effects on plant performance under low resource availability (Wise and Abrahamson 2005).

Deficits in soil water and nutrients would not always exacerbate belowground

herbivory damage because herbivores adjust their behavior in response to changes in water and nutrients in soil. Soil moisture often determines the survival of belowground herbivores and their abundance (Brown and Gange 1990; Villani and Wright 1990; Barnett and Johnson 2013). For instance, hatching rates of *Sitona lepidus* (Coleoptera: Curculionidae) and *Delia radicum* (Diptera: Anthomyiidae) decreased under drought conditions (Johnson et al. 2010; Lepage et al. 2012) and the survival of *Popillia japonica* larvae (Coleoptera: Scarabaeidae) declined under drought or excess moisture conditions (Fleming 1972; Crutchfield et al. 1995; Potter and Held 2002). Consequently, the damage from belowground herbivory on plant growth and survival should decrease under more severe conditions in soil.

There are no convergent perspectives on the effects of environmental conditions on plant-herbivore interactions (Hawkes and Sullivan 2001; Wise and Abrahamson 2005, 2007; Banta et al. 2010; Bagchi and Ritchie 2011) because knowledge on belowground herbivory is limited to the studies under resource rich environments.

Soil heterogeneity in water, nutrients and belowground plant-herbivore interactions

Essential resources for plants are heterogeneously distributed in space and time in soil (Robertson et al. 1988; Jackson and Caldwell 1993a, b; Cain et al. 1999; Farley and Fitter 1999; Kleb and Wilson 1999; James et al. 2003; Lundholm and Larson 2003). Nevertheless, the effects of heterogeneity on belowground plant-herbivore interactions

are rarely investigated because belowground herbivory has been mainly studied in agricultural systems, in which sufficient resources are supplied homogeneously. As resource heterogeneity in soil often determines plant competition and community dynamics (Hutchings and de Kroon 1994; Huber-Sannwald and Jackson 2001; Hodge 2004; McNickle et al. 2009), soil fauna and its feedback on plants have also strong influence on them (Plant-soil feedbacks; e.g., Kardol et al. 2006; Bardgett and Wardle 2010; Lankau et al. 2011). Therefore, soil fauna and resource heterogeneity synergistically affect plant dynamics (de Kroon et al. 2012).

In soil, the heterogeneity of nutrients and water could induce root distribution, and consequently, herbivores would follow the root. Many plant species proliferate their roots in nutrient-rich patches (Drew 1975; Hutchings and de Kroon 1994; Hodge 2004; Nakamura et al. 2008), inevitably causing root aggregation. Therefore, nutrient-rich patches are foraged by belowground herbivores because the herbivores graze roots more efficiently (Townsend et al. 2003), preferring both fine and fresh roots (van Dam 2009; Sun et al. 2011). Young roots of *Lolium perenne* were produced under a heterogeneous water supply in which roots increased 300-500% more (Jupp and Newman 1987).

The heterogeneous distribution of soil water and nutrients stimulates belowground herbivores to change their distribution. The behavior of belowground herbivores is altered by drought conditions (Staley and Johnson 2008; Barnett and Johnson 2013), in which drier soil causes belowground herbivores to move deeper into the soil profile. The type of herbivores includes grubs (Scarabaeidae; Villani and Wright 1988), carrot-fly larva (*Psila rosae* (Diptera: Psilidae); Jones 1979), and wireworms

(Elateridae; Lafrance 1968). However, irrigation causes grubs to inhabit more shallow areas in the soil profile (Villani and Wright 1988). Thus, the negative effects of belowground herbivory on plants are more severe under a homogeneous water supply, because belowground herbivores would occur and graze in shallow soil.

Hypotheses

I hypothesize that the reduced growth and survival of plants from belowground herbivory, depend on the vertical distributions of herbivores, the growth stages of plants, root architecture, and the heterogeneity of water and nutrients in soil profile.

The vertical distribution of a belowground herbivore

In Chapter 2, I hypothesize that the survival and biomass of a plant depend on vertical distribution of a belowground herbivore.

Plant growth stage and root architecture

In Chapter 3, I hypothesize: that plant mortality caused by belowground herbivory differ depending on the growth stages of plants and that forbs are more susceptible to belowground herbivory than grasses at early growth stages but grasses are more susceptible at late growth stages.

Heterogeneity of soil water and nutrients

In Chapter 4, I hypothesize that the amount of water and its heterogeneity alter the vertical distribution of belowground herbivores and their mortalities affecting plant growth.

In Chapter 5, I hypothesize that the effects of belowground herbivory on plant growth differ among plants with different root-foraging traits.

I conducted four garden experiments in microcosms to clarify the complex interactions between belowground herbivory and soil heterogeneity using the same species (Fig. 1-1, 1-2). The garden experiments focused on particular factors with obvious influences on plant growth and survival under controlled conditions (Fig. 1-3). Thus, garden experiments are currently one of the best options to understand interactions between belowground herbivory and plants (Townsend et al. 2003).

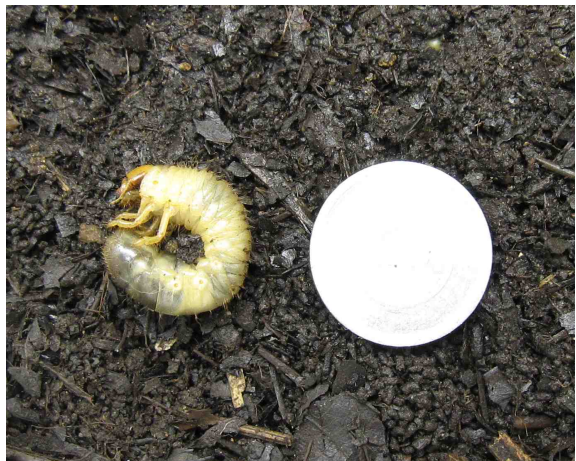
In General Discussion (Chapter 6), I discuss the changes in plant responses to belowground herbivory, because of the vertical distributions of belowground herbivores, plant growth stages, root architecture, and water and nutrients heterogeneity in the soil. I also mention the ecology of scarabaeid larvae and the defense strategies of plants on belowground herbivory.



(A)



(B)



(C)



(D)

Fig. 1-1. Scarabaeid beetles those were used in the experiments. (A) Adults of *Anomala cuprea* feeding upon *Fallopia japonica*; (B) Adults of Japanese beetle, *Popillia japonica* feeding upon *Trifolium pratense*; (C) A third-instar larva of *A. cuprea*; (D) A third-instar larva of *P. japonica*. A coin is 2 cm in diameter.



(A)

(B)



(C)



(D)

Fig. 1-2. Plants those were used in the experiments. (A) *Lolium perenne*; (B) *Plantago lanceolata*; (C) Fibrous root system of *L. perenne*; (D) Taproot system of *P. lanceolata*.

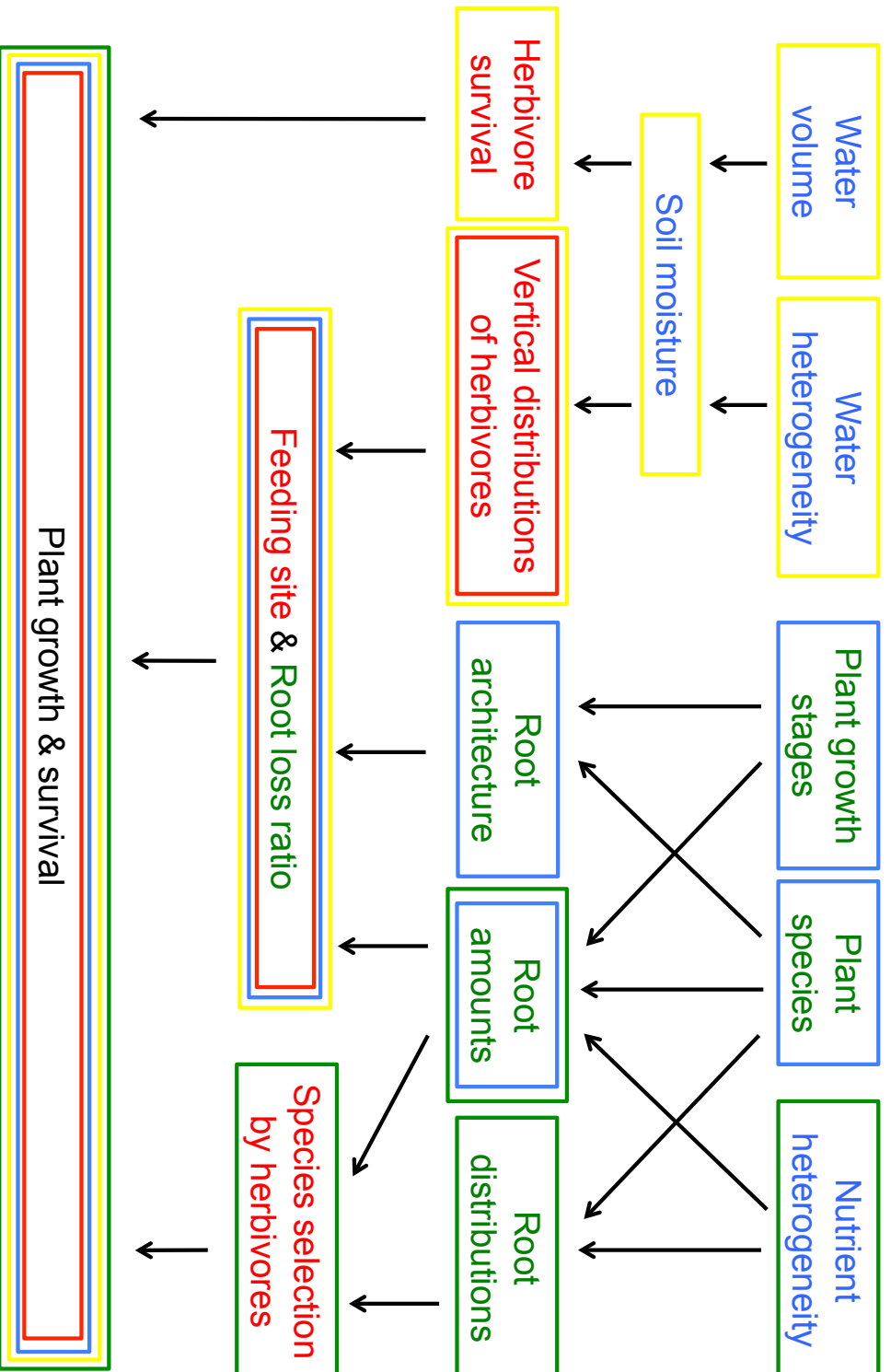


Fig. 1-3. A schema of the effects of belowground herbivory on plant growth and survival. Box colors mean target of chapters: red, Chapter 2; blue, Chapter 3; yellow, Chapter 4; green, Chapter 5. Font colors mean factors: red, belowground herbivores; blue, environmental factors, green, plants.

Effects of belowground vertical distribution of a herbivore on plant biomass and survival in *Lolium perenne*

Abstract

Root herbivory affects plant performance, but the effects are not well understood. We tested the effects of the vertical distribution of a root-feeding beetle larva (*Anomala cuprea*) by restricting its access to the top, middle, or bottom zone in pots of perennial ryegrass (*Lolium perenne*) or by allowing unrestricted access. We predicted that plant mortality, biomass, and biomass allocation should change with the zone of root herbivory, because both the magnitude of root loss and the consequences of such loss are specific to the point of damage. Seven of nine plants died in each treatment in which the larvae had access to the top zone. In contrast, no plants died when larvae occupied the middle or bottom zones. Plants were killed when the larvae grazed the root base and severed the shoots from the roots. Moreover, total plant biomass and biomass allocation to roots were significantly lower when the larvae were confined to the top and middle feeding zones. The greatest number of roots were removed when the larvae occupied the top feeding zone. Thus, the vertical distribution of a belowground herbivore is crucially important to plant fate. In nature, most belowground herbivores are concentrated near the soil surface, and thus the effects of belowground herbivory are often more severe than the effects of aboveground herbivory.

Keywords: Grub, Plant-herbivore interactions, Plant mortality, Plant-soil (belowground) interactions, Root herbivory

Introduction

Any given plant is vulnerable to several herbivore species (Crawley 1997), and plant interactions with aboveground herbivores have been extensively studied (Crawley 1983; Townsend et al. 2003; Schoonhoven et al. 2005). However, studies of belowground herbivory are less common (Andersen 1987; Brown and Gange 1990; Hunter 2001; Whittaker 2003; van Dam 2009), and its effects on plant performance are open to debate because experimental results conflict (see Zvereva and Kozlov 2012; Erb and Lu 2013). For example, Müller-Schärer (1991) initially reported that larvae of the root-boring moth *Agapeta zoegana* reduced the survival of spotted knapweed (*Centaurea maculosa*), but later found that root boring had no effect on survival (Steinger and Müller-Schärer 1992). The reason for this discrepancy is unclear. Nevertheless, belowground herbivores have been shown to be able to kill plants.

The most distinctive difference between aboveground and belowground herbivory is the effect on plant survival. Mature plants are rarely killed by aboveground herbivory (Crawley 1983; Ohgushi 2005), while belowground herbivory can be lethal: belowground herbivory by coleopteran larvae killed up to 30% of large *Oenothera erythrosepala* plants (Kachi 1983; Kachi and Hirose 1985). Strong et al. (1995) attributed sporadic and patchy mortality of mature bush lupines (*Lupinus arboreus*) to belowground herbivory by the ghost moth caterpillar *Hepialus californicus*.

Belowground herbivory by the crab *Sesarma reticulatum* increased mortality of the salt marsh plant *Spartina alterniflora* (Coverdale et al. 2012). Turf grasses infested by root-feeding grubs showed dead patches (Potter and Braman 1991). Thus, the life history consequences of plant damage caused by belowground herbivory can be more severe than those caused by aboveground herbivory (Maron 1998; Blossey and Hunt-Joshi 2003; Gerber et al. 2007; but see also Houle and Simard 1996; Zvereva and Kozlov 2012).

The removal of aboveground shoot biomass limits the growth, biomass allocation, survival, and fecundity of plants (e.g., Whigham 1990; Gustafsson 2004; Ishizaki et al. 2010). The amount of root removed also affects plant performance (Reichman and Smith 1991), and belowground herbivores often damage more root than they ingest (Erb and Lu 2013). Moreover, the amount of root removed should depend on the spatial distributions of the herbivores and their preferences for particular depths. If they graze in shallow soil, more root would be lost than if they graze more deeply, where roots are finer (Larcher 2003). Indeed, shoot and root biomass of *Lolium perenne* and *Trifolium repens* were lower in the presence of shallow-soil herbivores than in the presence of deep-soil herbivores (Davidson and Roberts 1969). Davidson and Roberts (1969) controlled the vertical distribution of belowground herbivores with mesh partitions, but because the depths of the partitions differed between the shallow and deep treatments, we cannot exclude the possibility that the partitions themselves contributed to the effects on the plants (e.g., McConnaughay and Bazzaz 1992). In addition, no plants died in their experiment, perhaps because the herbivores were not

able to graze the root base. To test plant damage under different patterns of herbivore vertical distribution, a different experimental design is necessary.

In natural habitats, most belowground herbivores occur in the top horizon of the soil. In one study of a subalpine grassland, for example, 85% of the belowground herbivores within the upper 30 cm of soil were found in the top 10 cm (Morón-Ríos et al. 1997). Significantly more larvae of the root-feeding weevil *Polydrusus sericeus* were sampled within the top 10 cm of soil than within the 10–20 and 20–30-cm layers in hardwood forests (Pinski et al. 2005). Thus, most belowground herbivory is expected to occur near the soil surface.

To better understand the effects of belowground herbivory on plants, we tested the following hypotheses. First, plant survival varies with the vertical distribution of belowground herbivores; specifically, we predicted that plant mortality would be higher if a belowground herbivore grazed in a shallower layer of the soil. Second, plant biomass varies as a function of the depth of the belowground herbivore, and thus the amount of root biomass lost should be greatest if a belowground herbivore occupies the shallowest soil depth; moreover, root loss diminishes root biomass and restricts root growth, so shoot biomass of grazed plants should be smaller. Finally, biomass allocation depends on the depth of belowground herbivores and, thus, affects root biomass.

Materials and Methods

In each experimental replicate, one plant of *L. perenne* was grown in potted soil with one third-instar larva (grub) of the beetle *Anomala cuprea* Hope (Coleoptera:

Scarabaeidae). The potted soil was vertically divided into three zones by stainless steel wire mesh that prevented vertical movement of the grub between zones. We evaluated the effects of the vertical distribution of the grubs on plant mortality, biomass, and biomass allocation.

Grubs were raised from eggs laid by adult *A. cuprea* that were collected on a floodplain of the Tama River in Tokyo, Japan, (35°38'N, 139°23'E) in June and July, 2010. The larvae typically feed on various herbaceous species (Okuno et al. 1978; Sakai and Fujioka 2007), including *L. perenne* (personal observation by T. Tsunoda).

The growth experiment was conducted in May and June, 2011, in a plastic film greenhouse under natural sunlight at the experimental garden of Tokyo Metropolitan University (Hachioji, Tokyo: 35°37'N, 139°23'E). Seeds of *L. perenne* were obtained from a commercial supplier (Takii & Co., Ltd., Kyoto, Japan) and germinated on granular red clay in Petri dishes. Ten days after germination, each plant at the first leaf stage was transplanted into a 20-cm × 20-cm × 18-cm plastic pot. Each pot was filled with 4 L of a 1:1 (v/v) mixture of granular red clay and black soil, and the recommended amount (20 g) of a slow-release fertilizer (Magamp K, 6 N : 40 P : 6 K : 15 Mg; Hyponex Japan, Osaka, Japan) was added to each pot. Pots were watered over the course of the experiment when the soil surface was dry.

The experiment used a one-way factorial randomized design with six treatments. In treatments 1, 2, and 3, one larva was confined by wire mesh partitions into the top, middle, or bottom zone of a pot, respectively. In treatment 4, no larva was introduced into a pot with partitions (negative-control treatment). In treatment 5, larval

movement was unrestricted in a pot with no partitions (positive-control treatment). In treatment 6, no larva and no partitions were used (plant-alone treatment). In treatments 1–4, two 20-cm × 20-cm stainless wire mesh partitions (5.5 mm mesh of 0.8-mm-thick wire) were placed in the soil so as to divide the soil evenly into three vertical zones that were each ca. 3 cm thick. In treatments 1–3, one grub was introduced into the designated zone through a small hole in the side of each pot, which was then sealed shut. In the positive-control treatment, four 20-cm × 10-cm wire mesh pieces were inserted down the side walls of the pot, and one grub was released on the top of the soil. Each treatment had nine replicates, and an additional five negative-control replicates were included to monitor plant growth, for a total of 59 pots.

Grubs were weighed individually and introduced into pots 20 d after transplanting, when the mean wet mass (\pm SD) of the grubs was 1.26 (\pm 0.13) g. Roots had reached the bottom zone in the negative-control treatment by this day. Plants were considered dead if the leaves were wilted and the root system was detached from the aerial parts. In the pots with dead plants, grub survival was recorded on the day the plants died.

Twenty days after the addition of grubs, all plants were harvested, and survival of grubs was recorded. The plants were divided into aerial parts and root systems, dried at 72°C for 3 days, and then weighed.

Data analysis

We compared plant mortality, biomass, and biomass allocation among treatments. All analyses were carried out with the statistical software R version 2.13.1 (R Development Core Team 2011). Plant mortality at harvest was analyzed by χ^2 test. We evaluated biomass data for homogeneity of variance using the Bartlett test and for normality using the Shapiro–Wilks test.

Plant biomass did not differ significantly between the negative control with partitions and the plant-alone treatments without partitions (*t*-test; mean plant biomass: $t_{16} = 0.659$, $P = 0.519$; mean shoot biomass: $t_{16} = 0.550$, $P = 0.590$; mean root biomass: $t_{16} = 0.943$, $P = 0.360$). Because there were no significant effects of wire mesh on biomass, we eliminated the plant-alone treatments from subsequent analyses.

We analyzed the effects of the vertical distribution of grubs on plant biomass, shoot biomass, and root biomass by one-way analyses of variance (ANOVAs). In these analyses, the response variable was biomass and the explanatory variables were the treatments and blocks. Significant differences between means were tested by Tukey's honestly significant difference (HSD) post-hoc test at $P < 0.05$. Dead plants were treated as missing values.

The effects of the vertical distribution of grubs on biomass allocation to roots were evaluated by analysis of covariance (ANCOVA) following Jasienski and Bazzaz (1999) and Müller et al. (2000). Root biomass was treated as the response variable and shoot biomass as the covariate (Murphy and Dudley 2007). The explanatory variables

were the treatments and blocks. The top-zone and positive-control treatments were excluded from ANCOVA because sample sizes were insufficient for the analysis.

Results

In both the top-zone and positive-control treatments, seven of nine plants died, but no plants died in the other treatments. Plant mortality differed significantly among treatments ($\chi^2 = 30.434$, $df = 4$, $P < 0.001$). All dead plants had been severed at the root base by the grubs.

Mean plant biomass varied significantly among treatments (ANOVA, $F_{4,18} = 5.876$, $P = 0.003$; Fig. 2-1). The mean biomass of plants was significantly larger in the negative-control treatment than in the top- and middle-zone treatments (Tukey's HSD). Grubs in the top zone significantly decreased the mean biomass of surviving plants; in contrast, grubs in the bottom zone had no significant effect on biomass (Tukey's HSD). Means of shoot and root biomasses also differed significantly among treatments (ANOVA; mean shoot biomass, $F_{4,18} = 4.119$, $P = 0.015$; mean root biomass, $F_{4,18} = 10.298$, $P < 0.001$; Fig. 2-1). The shoot biomass was significantly lower in the top-zone treatment than in the negative-control treatment (Tukey's HSD). The root biomass was significantly lower in the top-, middle-, and bottom-zone treatments than in the negative-control treatment (Tukey's HSD).

Biomass allocation to roots was affected by the feeding zone (Fig. 2-2). The covariate shoot biomass was significant (ANCOVA, $F_{1,13} = 83.721$, $P < 0.001$), which means that root biomass was significantly correlated with shoot biomass. The

correlation between root and shoot biomasses differed significantly among treatments (ANCOVA, $F_{2,13} = 19.683$, $P < 0.001$), which means that biomass allocation to roots differed significantly among treatments; in particular, the biomass allocation to roots was lower in the middle-zone treatment than in the bottom-zone treatment (Fig. 2-2).

At the end of the experiment, all grubs were collected alive from the zone into which they had been inserted. In the zones with grubs, detached roots were observed.

Discussion

Plants died only in the top-zone and positive-control treatments, because the larvae severed the shoots from the roots. Thus, plant fate depends on the vertical distribution of belowground herbivores.

When unrestricted, our belowground herbivores favored shallower soil, where they grazed on the base of the root system, as did other belowground herbivores in shallow soil (Morón-Ríos et al. 1997; Pinski et al. 2005). We found no differences in plant mortality between the top-zone treatment and the positive-control treatment, in which grubs moved without restriction. Belowground herbivory in shallow soil is ecologically significant because herbivores sever plant shoots from roots or detach roots altogether. Such dramatic root loss severely compromises the nutrient uptake capacity of the plant. Therefore, herbivory in shallow soil layers may damage plants more than grazing of the same biomass amounts from deeper in the soil or aboveground.

The effects of aboveground herbivory are often less profound than those of belowground grazing because leaf arrangement (Kikuzawa and Lechowicz 2011)

promotes herbivory of the terminal parts of a plant. In addition, grazed plants often compensate for the effects of leaf herbivory (Townsend et al. 2003).

The vertical distribution of belowground herbivores also affected biomass. Shoot growth decreased in the top-zone treatment, probably because the large loss of roots in this zone limited the absorption of water and nutrients. This result was consistent with that of Davidson and Roberts (1969). The plant biomass of the positive-control treatment differed clearly from that in the top-zone treatment (Fig. 2-1); however, the difference was not significant, probably because of the large variability and small sample size due to high mortality. The large variability in plant biomass reflected differences in the grazing points of the belowground herbivores (e.g., Robert et al. 2012).

The vertical distribution of belowground herbivores was crucially important in the allocation of biomass to roots. The allocation in the bottom-zone treatment was larger than that in the middle-zone treatment, perhaps because less root biomass was lost in the lower-zone treatment than in the middle-zone one.

The effects of belowground herbivory or artificial root removal on biomass allocation have been inconsistent among studies (e.g., Schmid et al. 1990; Steinger and Müller-Schärer 1992; Nötzold et al. 1998). The small biomass allocation to roots observed here may have been caused by the short growth period permitted after the addition of the grubs: the 20-d growth period was too short for the detection of enhanced root growth in this experiment, whereas 10 months allowed Steinger and Müller-Schärer (1992) to observe root regrowth and a decrease in the shoot-to-root

ratio.

This study experimentally showed that the effects of belowground herbivory on plant mortality, biomass, and biomass allocation vary depending on the soil depth at which the herbivory occurs. The damage was greatest when the herbivore could move in the shallowest soil layer. Belowground herbivores often occupy shallow soil layers (Morón-Ríos et al. 1997; Pinski et al. 2005) and, consequently, have more severe effects on plants than aboveground herbivores (Maron 1998; Blossey and Hunt-Joshi 2003; Gerber et al. 2007).

Funding

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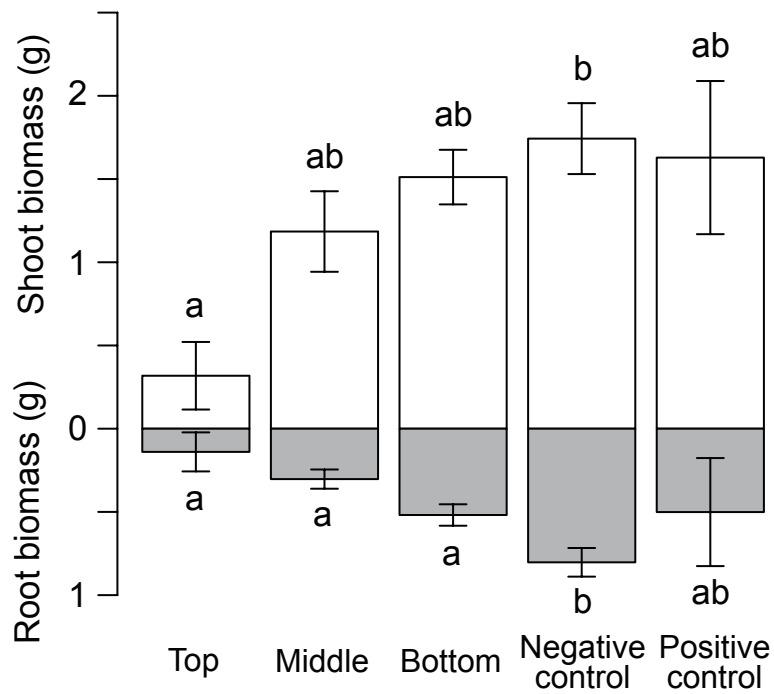


Fig. 2-1. Shoot and root mean biomass (\pm SE) after each of five feeding zone treatments.

Differences between bars with different letters are significant ($P < 0.05$, Tukey's HSD test).

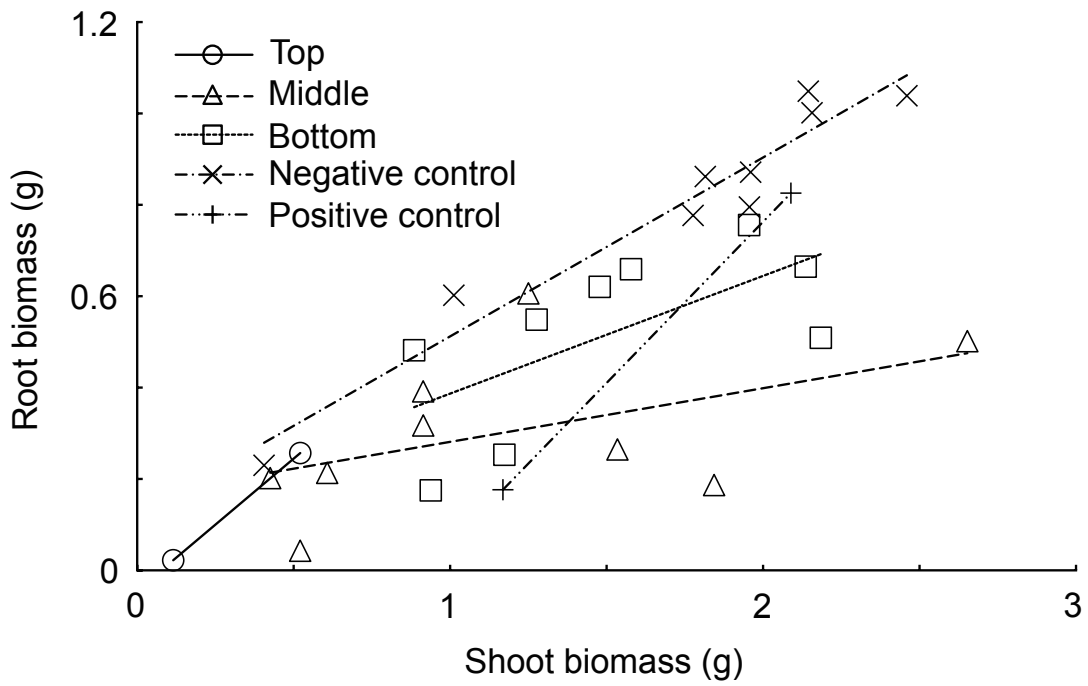


Fig. 2-2. Root versus shoot biomass in each of five feeding zone treatments. Linear regression lines are depicted.

**Effects of belowground herbivory on the survival and biomass of *Lolium perenne*
and *Plantago lanceolata* plants at various growth stages**

Abstract

We examined the effects of a root-feeding beetle larva (*Anomala cuprea* (Coleoptera: Scarabaeidae)) on plant survival and biomass at various growth stages in *Lolium perenne* and *Plantago lanceolata*. We predicted belowground herbivory often kills plants because of the large loss of roots at the earliest growth stages, but rarely at mature stages. We also predicted that *P. lanceolata* would exhibit higher mortality at the early growth stages than *L. perenne*, because the thin taproot of *P. lanceolata* is more susceptible to herbivory, and biomass reduction owing to herbivory at the late growth stages would occur only in *L. perenne*. Plants of *L. perenne* or *P. lanceolata* at four different growth stages were grown in pots with or without a herbivore. Herbivores killed plants at the early growth stages in both species but killed no plants at the last stage. In *P. lanceolata*, large biomass loss at the early growth stages was attributed to herbivory, but no loss in biomass was observed at the last stage. In contrast, herbivory reduced biomass of *L. perenne* at all growth stages. Our results strongly show that the effects of belowground herbivory depended on growth stages and root architecture of plants.

Keywords: Grubs, Plant–soil interactions, Root architectures, Root herbivore

Introduction

Belowground herbivores significantly shape many ecosystem processes, such as plant successional patterns (Brown and Gange 1989, 1992; De Deyn et al. 2003; Rasmann et al. 2011); this occurs because belowground herbivory causes more severe damage to plants than aboveground herbivory (Maron 1998; Blossey and Hunt-Joshi 2003; Gerber et al. 2007; but see Zvereva and Kozlov 2012), and even occasionally causes plant mortality (Kachi 1983; Kachi and Hirose 1985; Strong et al. 1995; Coverdale et al. 2012; Tsunoda et al. in press). Belowground herbivory, therefore, determines the fate of some plants as well as influences community dynamics (Gange and Brown 2002; van der Putten 2003; Stein et al. 2010). However, we have limited knowledge related to belowground herbivory compared with that on aboveground herbivory (Hunter 2001; Whittaker 2003; van Dam 2009).

Plants at different growth stages should experience different effects from herbivory. The risks and damage from aboveground herbivory differ as plants enter different growth stages (Strauss and Agrawal 1999; Boege and Marquis 2005; Hanley and Fegan 2007; Hanley et al. 2013). For example, seedlings are the most vulnerable life stage and are susceptible to death from aboveground herbivory; the loss of small fractions of tissues can cause mortality at the early growth stages (Moles and Westoby 2004; Fenner and Thompson 2005; Hanley and May 2006; Hanley and Sykes 2009). However, Stout et al. (2002) provided almost the only extant report related to the tolerance of rice

plants at different growth stages to a belowground herbivore, *Lissorhoptrus oryzophilus* Kuschel. Therefore, we have only limited knowledge on changes in belowground-herbivory relationships with plant growth.

Root architecture has various effects on herbivory at different growth stages. For example, root herbivory should be fatal to most forbs at the early growth stages because root architecture consists of a thin taproot and lateral branch roots, although at mature stages root architecture includes a well-developed taproot. In contrast, many grass species have vegetative root branches at the base of their shoots (Kays and Harper 1974) so that root architecture does not change between the various growth stages. Thus, root herbivory likely causes more or less similar effects on grass species at all growth stages. Therefore, different species with contrasting root architecture should exhibit different response patterns to belowground herbivory (Mortimer et al. 1999) during growth.

In this experiment, we evaluated the effects of belowground herbivory on plant survival and biomass at various growth stages of a grass, *Lolium perenne* L., with a fibrous root system and a forb, *Plantago lanceolata* L., with a taproot system. We proposed two hypotheses. First, plant mortality caused by belowground herbivory differs depending on the growth stages of a plant. We predicted that belowground herbivory often kills plants when most of the roots are lost at the earliest growth stages, but it rarely kills plants at mature stages. Second, forbs are more susceptible to belowground herbivory than grasses at the early growth stages but less at late stages: even slight herbivory destroys thin taproot systems but not well-developed ones. On the contrary, grasses with fibrous root systems are subject to certain damages from herbivory that

diminish their biomass. We predicted that the forb, *P. lanceolata*, will exhibit higher mortality at early growth stages than the grass, *L. perenne*, and a reduction in biomass at the late stages will occur only in *L. perenne*.

Materials and Methods

Study species

One plant of *Lolium perenne* L. or *Plantago lanceolata* L. was grown in each of several pots, and a third-instar larva of the generalist root-chewing insect *Anomala cuprea* Hope (Coleoptera: Scarabaeidae, hereafter ‘grub’) was also added to some of the pots. *L. perenne* and *P. lanceolata* often coexist in seminatural grasslands and pastures. Seeds of *L. perenne* cv. *amazing* were obtained from a commercial supplier (Snow Brand Seed Co., Ltd, Sapporo, Japan). Seeds of *P. lanceolata* were collected from a floodplain of the Ohta River in Tokyo (35°37'N, 139°23'E) during August 2009. Grubs of *A. cuprea* feed on various herbaceous species (Okuno et al. 1978; Sakai and Fujioka 2007), including *L. perenne* and *P. lanceolata* (T. Tsunoda, personal observation). The grubs used in this experiment were grown from eggs laid in humus by adults *A. cuprea* collected from a floodplain of the Tama River in Tokyo (35°38'N, 139°23'E) in summer 2009.

Experimental design

We conducted the experiment from August to October 2009 in a plastic film greenhouse under natural sunlight in the experimental garden of Tokyo Metropolitan University (Hachioji, Tokyo: 35°37'N, 139°23'E). Plants were grown in 14-cm diameter and

18-cm-deep plastic pots, each filled with 1200 ml of a mix of granular red clay and black soil (ratio 1:1 v/v), and 4 g of slow-release fertilizer (Magamp K, 6:40:6:15 (N-P-K-Mg), Hyponex Japan, Osaka, Japan) at half the recommended amount. Seeds of both species were sown on wet quartz sand and grown in a growth chamber (Koito PC-02, Koito Industries, Ltd., Kanagawa, Japan) with a 14-h day/10-h night cycle at a temperature of 25°C. Seven days after sowing, one seedling was transplanted in each pot.

The growth experiment had a three-way full factorial randomized block design with 15 replications: the factors were plant species, belowground herbivore, and growth stage. The belowground herbivore treatment had two levels: presence or absence of a grub in a pot. The plant growth stage treatment had four levels: one seedling was transplanted into each pot on September 20 for the 15-days treatment before herbivory occurred, September 10 for the 25-days treatment, August 31 for the 35-days treatment, and August 21 for the 45-days treatment. The pots received water daily.

Each weighed grub introduced near the center of the soil surface, dug itself beneath the soil surface on October 5. The mean fresh weights of grubs did not differ significantly between plant species and growth stage (two-way analysis of variance, plant species, $F_{1,102} = 0.355$, $P = 0.553$; growth stage, $F_{1,102} = 0.012$, $P = 0.914$; their interaction $F_{1,102} = 0.030$, $P = 0.862$). Plants were considered dead if the leaves were wilted and the root system was detached from the aerial parts. We recorded the survival of grubs in the pots on the day when the plants died.

On day 25 after the introduction of grubs, we harvested plants and divided them into shoots and roots, dried the shoots and roots separately at 70°C for 3 days and then

weighed them. Plant biomass equals the sum of the shoot and root biomass of each plant. At the harvest, we recorded the survival or death of grubs and weighed living grubs individually.

Statistical analyses

Plant mortality and plant biomass, as well as mortality and fresh weight of belowground herbivores, were compared between treatments. All analyses were carried out with the statistical software R version 2.15.1 (R Development Core Team, 2012). Plant biomass and fresh weight of belowground herbivores were evaluated for homogeneity of variance and normality using the Bartlett and Shapiro-Wilks tests, respectively.

Mortalities of plants and grubs were analyzed with a generalized linear mixed model (GLMM) assuming a binomial error distribution (Crawley 2007). The model of plant mortality used plant mortality as the response variable and plant species, growth stage, belowground herbivore, and their interactions as the explanatory variables with fixed factors; the random factor was block. The model of belowground herbivore mortality used belowground herbivore mortality as the response variable, and plant species, growth stage, and their interaction as the explanatory variables with the fixed factors; the random factor was block. The likelihood ratio test using a chi-square test statistic allowed comparisons of the models with and without the explanatory variables (Crawley 2007).

Plant biomass was analyzed with GLMMs assuming a Gaussian error distribution. This model used biomass as the response variable and plant species, growth

stage, belowground herbivory, and their interactions as the explanatory variables with the fixed factors; the random factor was block. For each plant species, biomass was also analyzed as the response variable; plant growth stage, belowground herbivory, and their interaction were the explanatory variables with the fixed factors; the random factor was block. In the plant biomass analyses, if the assumption of homogeneity of variance and normality were satisfied, then an identity-link function was applied. If not, then a log-link function was applied. Data from pots in which belowground herbivores or plants died were treated as missing values. We used type-II Wald tests using a chi-square test statistic to determine the effects of the fixed factors.

The effects of plant species and growth stage on the fresh weight of belowground herbivores were evaluated by analysis of covariance (ANCOVA). Fresh weight after the experiment was treated as the response variable and fresh weight before the experiment as the covariate. The explanatory variables were plant species, growth stage, and block.

Results

No experimental treatments significantly affected mortality of the belowground herbivore (plant species, $\chi^2 = 2.661$, $df = 1$, $P = 0.103$; growth stage, $\chi^2 = 1.528$, $df = 3$, $P = 0.676$; their interaction $\chi^2 = 1.649$, $df = 3$, $P = 0.648$), and its fresh weights (Table 3-1). Plant mortality significantly depended on plant species ($\chi^2 = 4.011$, $df = 1$, $P = 0.044$), growth stage ($\chi^2 = 69.944$, $df = 3$, $P < 0.001$), and belowground herbivory ($\chi^2 = 69.979$, $df = 1$, $P < 0.001$). Plant mortality only occurred in the treatments with a belowground herbivore.

In *P. lanceolata*, 14 plants died in the 15-days treatment, six in 25-days treatment, and one in 35-days treatment. In *L. perenne*, 10 died in 15-days treatment and four in 25-days treatment (Fig. 3-1). No plant died in *P. lanceolata* in the 45-days treatment and in *L. perenne* in the 35- and 45-days treatments.

The complicated interactions between experimental treatments altered mean plant biomass (Fig. 3-2). All three of the possible two-way interactions, plant species and growth stage ($\chi^2 = 24.560$, $df = 3$, $P < 0.001$), belowground herbivory and plant species ($\chi^2 = 25.628$, $df = 1$, $P < 0.001$), and belowground herbivory and growth stage ($\chi^2 = 15.616$, $df = 3$, $P = 0.001$), significantly affected mean plant biomass.

Mean plant biomass of *P. lanceolata* was significantly affected by the two-way interaction between belowground herbivory and growth stage ($\chi^2 = 15.064$, $df = 3$, $P = 0.002$; Fig. 3-2A), which means the effects of belowground herbivory differed at different growth stages of *P. lanceolata*. Mean plant biomasses increased along with growth stages. In the 15-, 25-, and 35-days treatments, plants with a belowground herbivore had a smaller mean plant biomass than those without a herbivore, but in the 45-days treatment, belowground herbivory did not influence mean plant biomass (Fig. 3-2A). Herbivores did not sever the thick taproots of *P. lanceolata* at the late growth stages but did detach their lateral roots (Fig. 3-3A).

Mean plant biomass of *L. perenne* significantly varied at different growth stages ($\chi^2 = 231.359$, $df = 3$, $P < 0.001$; Fig. 3-2B) and that varied significantly in the belowground herbivory treatments ($\chi^2 = 51.787$, $df = 1$, $P < 0.001$). Mean plant biomass of *L. perenne* increased at the late growth stages, and that in the presence of a

belowground herbivore were smaller than those without at all growth stages (Fig. 3-2B). Roots of *L. perenne* had the same morphology at all growth stages, and belowground herbivores often detached many roots at the late growth stages (Fig. 3-3B). Those herbivores also detached some tillers from the roots of *L. perenne* at the late stages and the tillers wilted.

Discussion

Both *P. lanceolata* and *L. perenne* experienced high mortality at the early growth stages, probably because belowground herbivores almost completely grazed the small available roots. This result revealed that belowground herbivory proved fatal in the same way that aboveground herbivory generally results in fatality during the early growth stages (Moles and Westoby 2004; Fenner and Thompson 2005). The fact that no mortalities occurred in both species at mature growth stages can be attributed to the relatively small decrease of intact root biomass caused by grazing. In mature plants, the amounts grazed by a grub would be more or less constant because the fresh weights of belowground herbivores remained more or less constant between treatments.

Root architecture probably caused the higher mortality observed in *P. lanceolata*. Belowground herbivory likely severs the single, thin taproot of *P. lanceolata* at the early growth stages, and juvenile plants would often lose entire root systems. In contrast, *L. perenne* has several individual roots even at the very early growth stages (Kays and Harper 1974), which would mitigate damages by herbivores. Our results

demonstrate that plant susceptibility to belowground herbivory depends on root architecture (Mortimer et al. 1999).

The loss of root biomass observed in *P. lanceolata* was attributed to belowground herbivory; as taproot and lateral root developed, the percentages of tissue lost to herbivory declined. Some belowground herbivores prefer fine lateral roots to taproots (Sun et al. 2011) and would not select the very tough well-developed taproots found at the late growth stages.

Grazing points determine the effects of belowground herbivores on plants (Robert et al. 2012; Tsunoda et al. in press). For example, root biomass of *Asclepias syriaca* was lost by the *Tetraopes tetraophthalmus* larvae to feed on a taproot, but not by click beetle larvae to graze on fine roots (Erwin et al. 2013).

Belowground herbivory on the fibrous roots of *L. perenne* reduced the plant biomass at all growth stages. Even a mature and large *L. perenne* plant produces fibrous root modules, allowing it to increase root biomass (Kays and Harper 1974), and each root would have equal risk of being severed. In addition, belowground herbivores would also graze basal meristems (Haukioja and Koricheva 2000). Therefore, belowground herbivory would impair biomass increment of *L. perenne* at all growth stages.

Plant mortality caused by belowground herbivory differed at different growth stages supporting our first hypothesis; this also resembles observations of aboveground herbivory (Strauss and Agrawal 1999; Nykänen and Koricheva 2004; Boege and Marquis 2005; Hanley et al. 2013). Our results that show *P. lanceolata* was more susceptible to belowground herbivory than *L. perenne* at the early growth stages are consistent with our

second hypothesis. However, these results are inconsistent with a recent meta-analysis (Zvereva and Kozlov 2012) showing that belowground herbivory on mature plants affects grasses more profoundly than forbs. This inconsistency is ascribed to the experimental designs of previous studies in which herbivory was examined at a particular growth stage, with an exception of Stout et al. (2002) that studied herbivory at various growth stages. Our study strongly shows that effects of belowground herbivory on plants differ depending on the growth stage and on root architecture. Therefore, in the future we should investigate the effects of belowground herbivory on plants and in communities at various growth stages, because plants in communities exhibit diverse growth patterns and root architectures.

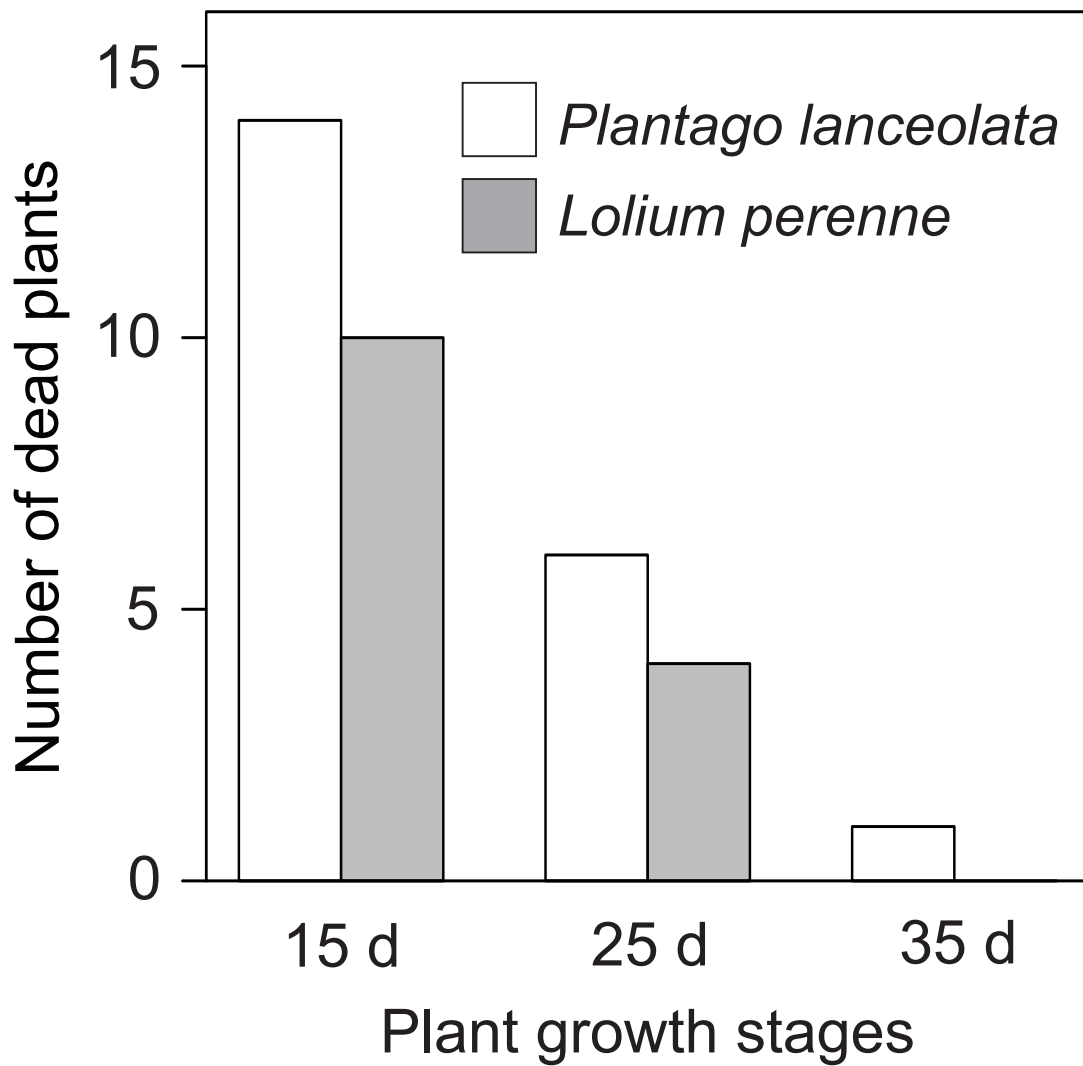


Fig. 3-1. Mortality of *Plantago lanceolata* and *Lolium perenne* in the presence of a belowground herbivore. *L. perenne* at 35-days treatment and both plant species at the 45-days treatment suffered no mortality.

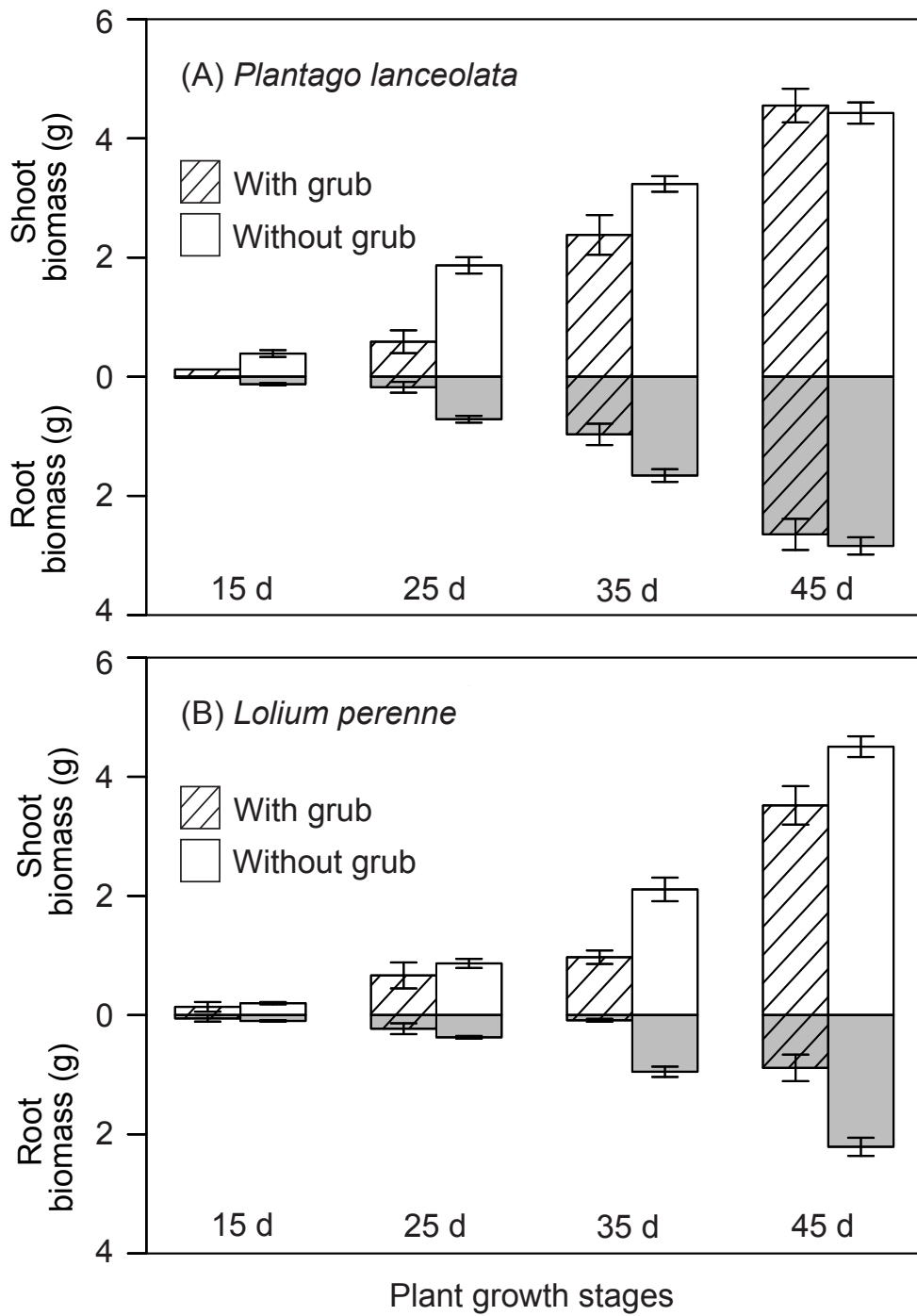


Fig. 3-2. Shoot (open bars) and root (gray bars) mean biomass (\pm SE) after each growth period and belowground herbivore treatment. (A) *Plantago lanceolata*; (B) *Lolium perenne*.



(A)



(B)

Fig. 3-3. Root system grazed on by a larva of *Anomala cuprea*. (A) Taproot system of *Plantago lanceolata*; (B) Fibrous root system of *Lolium perenne*. The circle within the figures points to grazed point.

Table 3-1. Analysis of covariance to test the effects of grub fresh weight before the experiment (W), plant species (P), growth stage (G), and block on grub fresh weight after the experiment.

Source	SS	df	F	P
W	0.217	1	26.945	< 0.001
P	0.015	1	1.864	0.182
G	0.051	3	2.098	0.122
W × P	0.010	1	1.210	0.280
W × G	0.012	3	0.508	0.680
P × G	0.039	3	1.602	0.210
W × P × G	0.009	2	0.524	0.597
Block	0.175	14	1.549	0.153
Residuals	0.242	30		

Availability and temporal heterogeneity of water supply affect the vertical distribution and mortality of a belowground herbivore and consequently plant growth

Abstract

We examined how the volume and temporal heterogeneity of water supply changed the vertical distribution of a belowground herbivore and consequently affected plant growth. *Plantago lanceolata* (Plantaginaceae) seedlings were grown at one per pot under different combinations of water volume (large or small volume) and heterogeneity (homogeneous water conditions, watered every day; heterogeneous conditions, watered every 4 days) in the presence or absence of a larva of the belowground herbivorous insect, *Anomala cuprea* (Coleoptera: Scarabaeidae). The larva was confined in different vertical distributions to top feeding zone (top treatment), middle feeding zone (middle treatment), or bottom feeding zone (bottom treatment); alternatively no larva was introduced (control treatment) or larval movement was not confined (free treatment). With a large water volume and free treatment, plant biomass was low regardless of heterogeneity (similar to that under top treatment). With a small water volume and free treatment, plant biomass was low (similar to that under top treatment) under homogeneous water conditions but high under heterogeneous ones

(similar to that under middle or bottom treatment). Herbivore mortality was high with heterogeneous application of a small water volume or homogeneous application of a large volume. With a large water volume, plant biomass was high in pots in which the herbivore died. With heterogeneous supply of small-volume water, herbivores would distribute deep in the soil to avoid the dry surface soil. Therefore, there was little effect on plant growth. In other watering regimes, herbivores distributed in the shallow soil and reduced root biomass. The combinations of water volume and heterogeneity affected plant growth via the change of a belowground herbivore.

Keywords: Heterogeneous water supply, Plant–herbivore interaction, Plant–soil (belowground) interaction, Root herbivory, Soil moisture

Introduction

Water availability and its temporal variability (hereafter, ‘water heterogeneity’) affect plant biomass growth (Novoplansky and Goldberg 2001; Hagiwara et al. 2008). Plant responses to water frequency vary depending on nutrient availability and soil water content (Maestre and Reynolds 2007; Heisler-White et al. 2008; Knapp et al. 2008; Hagiwara et al. 2010). Soil water status also affects soil biota (Bardgett 2005), and interacts with soil biota to affect plant growth. Empirical studies are necessary to clarify the interactive effects of water heterogeneity on soil biota and hence on plant growth because of the important influence of soil biota on plant growth and

community dynamics (Bardgett 2005; Bardgett and Wardle 2010).

In light of the presence of herbivorous insects in the soil, it is important that we elucidate the effects of water availability and heterogeneity on plants (Staley and Johnson 2008), because these insects influence the abundance, species diversity, and succession of plants (Brown and Gange 1992; De Deyn et al. 2003; Wardle et al. 2004; van der Putten et al. 2009; Stein et al. 2010). Erb and Lu (2013) pointed out that heterogeneity of soil abiotic factors such as moisture and nutrient availability alters the effects of belowground herbivores on plants. The interactions between water availability, heterogeneity, and belowground herbivory are likely to play crucial roles in plant growth.

Water availability and frequency affect the vertical distribution of belowground herbivores, and thus plant growth, because soil insects move vertically in response to changes in soil water status (Villani and Wright 1990; Barnett and Johnson 2013). Grubs (Coleoptera: Scarabaeidae) are distributed deep in the soil in response to drought and shallow in the soil in response to irrigation (Villani and Wright 1988). The carrot-fly larva *Psila rosae* feeds on roots 15 cm below the ground in semi-dry soil, whereas in moist soil it feeds 1 cm from the soil surface (Jones 1979). Wireworms (Coleoptera: Elateridae) are distributed deep in the soil in summer but in shallow soil after heavy rain (Lafrance 1968). These changes in the vertical distribution of belowground herbivores affect plant mortality and growth (Davidson and Roberts 1969; Tsunoda et al. in press). Therefore, the amount and heterogeneity of water

supply that determine soil moisture levels are likely to affect the vertical distribution of belowground herbivores and thus plant growth.

Soil moisture is one of the most important factors affecting the survival and abundance of belowground herbivorous insects (Brown and Gange 1990; Villani and Wright 1990; Barnett and Johnson 2013). Soil dryness increases mortality of belowground herbivores (Campbell 1937; Moran and Whitham 1988; Briones et al. 1997; Riis and Esbjerg 1998), whereas in moist soil mortality either does not change (Ladd and Buriff 1979; Régnière et al. 1981; Moran and Whitham 1988) or increases (Campbell 1937; Godfrey and Yeargan 1985). These findings suggest that the mortality of belowground herbivores in response to extreme water events has the potential to affect plant growth in various ways.

We conducted a growth experiment to test the hypothesis that changes in the amount and heterogeneity of water supply alter the vertical distribution and mortality of belowground herbivores and thus affect plant growth. Under heterogeneous supply of small amount water, belowground herbivores will become distributed deep in the soil to avoid the dry surface soil and feed on the fine root tips, which will hardly affect plant growth. In contrast, under homogeneous supply of large volume of water, herbivores will become distributed shallow in the soil and detach root connection by grazing, which has impact on plant growth. Soil water status resulting from changes in water supply amount and heterogeneity will also determine the fate of belowground herbivores. Unless belowground herbivores are present, plant growth will be no longer

restricted by belowground herbivory.

Materials and Methods

Study species

One seedling of *Plantago lanceolata* L. (Plantaginaceae) was grown in each pot, to which we added a third-instar larva, or grub, of the belowground herbivorous insect *Anomala cuprea* Hope (Coleoptera: Scarabaeidae). The short-lived perennial forb, *P. lanceolata*, is cosmopolitan and has a rosette growth form. Seeds of *P. lanceolata* were collected from a population of more than 30 plants on a floodplain of the Tama River in Tokyo (35°38'N, 139°23'E). *P. lanceolata* is not endangered or protected species, and no specific permissions were required for this location to collect seeds of *P. lanceolata*. Larvae of *A. cuprea* feed on various herbaceous species (Okuno et al. 1978; Sakai and Fujioka 2007), including *P. lanceolata* (T.Tsunoda, personal observation). Grubs were grown from eggs laid in humus by adult *A. cuprea* collected from a floodplain of the Tama River (35°38'N, 139°23'E) in June and July 2012. *A. cuprea* is not endangered or protected species, and no specific permissions were required for this location to collect insects.

Experimental design

The growth experiment was conducted from September to October 2012 in a plastic film greenhouse under natural sunlight in the experimental garden of Tokyo

Metropolitan University (Hachioji, Tokyo; 35°37'N, 139°23'E). The mean annual precipitation in Hachioji has been 1602.3 mm year⁻¹ over the past 30 years (Japan Meteorological Agency). Seeds of *P. lanceolata* were sown in a tray of peat moss in a growth chamber (Koito PC-02, Koito Industries, Ltd., Kanagawa, Japan) at 25°C. Seven days after sowing, one seedling with cotyledons was transplanted into each plastic pot (20 × 20 × 18 cm deep). Each pot was filled with 4.8 L of a mix of granular red clay and black soil (ratio 1:1 v/v) with 16 g of slow-release fertilizer [Magamp K, 6:40:6:15 (N-P-K-Mg), Hyponex Japan, Osaka, Japan].

The experiment had a three-way factorial randomized block design with nine replications. The factors were water volume with two levels (large, 200 mL of water per day, and small, 100 mL of water per day), water supply frequency with two levels (homogeneous, daily watering, and heterogeneous, watering every 4 days), and belowground herbivore. The maximum amount of water given at any one time did not exceed the capacity of the pot. The total volume of water received over the period was the same between the homogeneous and heterogeneous supplies for each water volume regime.

There were five types of belowground herbivore treatment: in the top treatment, one grub was placed in the top zone; in the middle treatment, it was placed in the middle zone; and in the bottom treatment, it was placed in the bottom zone. In the control treatment, no grub was added to the pot. In the free treatment, no screens were added to restrict the grub movement. Two 20 × 20 cm stainless-steel wire-mesh

screens (0.8-mm wire diameter, 5.5-mm mesh) were inserted into the pot soil to divide the soil evenly into three zones in all treatments except the free treatment. In the top, middle, and bottom treatments, one grub was introduced to the relevant zone through a hole (diameter 12 mm) on the side wall of the pot. The hole was closed with plastic film after the grub had been added. In the free treatment, four screens of 20 × 10-cm stainless-steel wire-mesh were inserted, one along each wall of the pot. Thus, the grub was able to move freely around the pot.

For the first week after transplantation of the seedlings, 150 mL water was supplied to all pots every day. The treatments that combined different water volumes with different watering frequencies began at the start of the second week after the transplant. On day 20 after the beginning of the watering treatment, a grub was added to the relevant zone of the pot. In the free treatment, the grub was placed on the soil surface near the centre of the pot and left to burrow underground.

A plant survival was recorded every day after addition of the grub. If the roots became completely detached from the shoot and the leaves wilted, the plant was considered to die. When a plant died, we recorded survival of the grub was.

On day 28 after addition of the grub, the plants were harvested and divided into shoots and roots. The shoots and roots were dried at 72°C for 3 days and weighed. At harvest, grub mortalities were also recorded.

Soil moisture measurement

Soil moisture (water content by volume) was measured with a soil moisture probe (ECH₂O, Decagon Devices, Inc., Pullman, Washington, USA) in an additional four replications without grubs of each watering treatment combination and soil zone during the experimental period. Measurements were taken every day, before the watering. Relative soil moisture content was calculated as the difference between the measured value and the minimum value during the experimental period, divided by the range between the maximum and minimum values during the experimental period (James et al. 2003). The 4-day moving variance of the relative soil moisture content for the current day and the next 3 days was calculated to quantify the variability in water availability in each 4-day watering cycle. The coefficient of variation (CV) and temporal mean of the relative soil moisture content during each watering treatment were calculated. The CV was used as an index of temporal variability in soil moisture content during each watering treatment (James et al. 2003).

Data analysis

Relative soil moisture content and temporal variability in soil moisture were analyzed by using generalised linear mixed models (GLMMs) under the assumption of a Gaussian error distribution. In this model, the response variable was the relative soil moisture content or the CV; the explanatory variables were water volume, watering frequency, soil zone, and their interactions: the random factors were measuring date

and block, with block as nested random effect within date.

Mortalities of plants and grubs were analyzed by using GLMMs with a binomial error distribution and logit-link function. In these models, the response variable was grub or plant mortality; the explanatory variables were water volume, watering frequency, belowground herbivore, and their interactions. The random factor was block.

Plant biomass was analyzed with a GLMM assuming a Gaussian error distribution. In this model, the response variable was plant biomass; the explanatory variables were water volume, watering frequency, belowground herbivore, and their interactions. The random factor was block. For each water volume, plant biomass was analyzed with the model lacking water volume as an explanatory variable.

In the analyses for continuous variables, if the assumption of homogeneity of variance was satisfied with Bartlett's test, then identity-link function was applied. If not, then log-link function was applied. Data from pots in which belowground herbivores died were treated as missing values.

Because several grubs were found dead at harvest, plant biomass was analyzed with generalised linear models (GLMs) for each water volume to evaluate changes in plant biomass due to herbivore mortality. In these models, the response variable was plant biomass; the explanatory variables were watering frequency, belowground herbivore, the survival of the belowground herbivore, and their interactions.

All analyses were performed with the statistical software R version 2.15.1 (R Development Core Team 2012). The lme4 package was used to calculate GLMMs by using maximum likelihood estimation. To determine the effects of the fixed factors, we used a likelihood ratio test to compare models with and without the variable of interest using a chi-squared test statistic (Crawley 2007). The data were analyzed by GLMM framework because our data contain binary and continuous variables (Bolker et al. 2008).

Results

Soil moisture

The mean relative soil moisture content was larger under a large water volume than a small volume (Fig. 4-1, $\chi^2 = 38.788$, $df = 1$, $P < 0.001$). The mean relative soil moisture content in the bottom zone was the largest, and that in the top zone was the smallest (Fig. 4-1; $\chi^2 = 6.048$, $df = 2$, $P = 0.049$).

The temporal variability in relative soil moisture content under heterogeneous water-supply conditions was larger than that under homogeneous conditions (Fig. 4-1). The mean CV (%) of the relative soil moisture content was larger under heterogeneous water-supply conditions than under homogeneous conditions ($\chi^2 = 6.425$, $df = 1$, $P = 0.011$).

Plant mortality

Plant mortality changed significantly with differences in the feeding zones of the belowground herbivore ($\chi^2 = 8.621$, $df = 3$, $P = 0.035$). No plants died in the middle and bottom treatments, but three died in the top treatment and three in the free treatment. No plants died when a small volume of water was heterogeneously applied (Table 4-1).

Plant biomass

The three-way interaction between water volume, watering heterogeneity and belowground herbivory, significantly affected mean plant biomass (Fig. 4-2, $\chi^2 = 27.610$, $df = 4$, $P < 0.001$). The herbivory effects on plant biomass differed between the combinations of water-supply volume and heterogeneity: belowground herbivory interacted with heterogeneous conditions and a small water volume (Fig. 4-2A; $\chi^2 = 32.501$, $df = 4$, $P < 0.001$). When a small water volume was supplied, the plant biomass was larger in the control treatment than in the free treatment under homogeneous conditions and smaller than in the free treatment under heterogeneous conditions (Fig. 4-2A). Under both homogeneous and heterogeneous conditions, plant biomass in the top treatment was the smallest between the three zone treatments. Plant biomass in the free treatment was nearly the same as that in the top treatment with a homogeneous water supply and as large as that in the middle or bottom treatment with a heterogeneous water supply.

Under water supply of large volume, plant biomass significantly differed depending on the vertical distribution of a belowground herbivore (Fig. 4-2B; $\chi^2 = 20.968$, $df = 4$, $P < 0.001$). In the treatment with herbivore in the top zone, plant biomass was remarkably small under both water heterogeneity conditions. Plant biomass in the free treatment was the smallest in all treatments with a homogeneous water supply, and plant biomass in the free treatment was slightly larger than in the top treatment with a heterogeneous water supply.

Mortality of belowground herbivores and its effect on plant biomass

The interaction between water volume and water supply heterogeneity significantly affected mortality of belowground herbivores ($\chi^2 = 5.249$, $df = 1$, $P = 0.022$). With a large water volume, 15 belowground herbivores in the homogeneous treatment died, as did 9 in the heterogeneous treatment. With a small water volume, 5 died in the homogeneous treatment and 11 in the heterogeneous one. Mortality of belowground herbivores did not differ between the feeding zones ($\chi^2 = 0.895$, $df = 3$, $P = 0.827$).

Mortality of the belowground herbivore significantly affected plant biomass with a large water volume (GLM, $F = 6.793$, $df = 1$, $P = 0.012$) but not with a small water volume (GLM, $F = 0.321$, $df = 1$, $P = 0.573$). Plant biomass in the pots with herbivore mortality was larger than those without mortality.

Discussion

Plant mortality occurred only in the treatments in which the herbivore was shallow in the soil to sever the aerial shoot from its root system. This plant mortality caused by belowground herbivory is consistent with our previous study (Tsunoda et al. in press). With heterogeneous supply of small volume water, no plant was grazed at the root base and any plants did not die, probably because low moisture in the surface soil was not suitable for grubs to graze there.

The interaction between available volume and heterogeneity of water supply would change the vertical distribution of the grub in the free treatment. With a small volume of water under a heterogeneous supply, the mean plant biomass in the free treatment that was almost equivalent to those in the bottom and middle treatments, suggest the grubs occurred in the bottom and middle zones due to the low soil moisture levels and large moisture variability. Consequently, the herbivory effects on plant growth varied depending on the availability of water supply.

The vertical distribution of the belowground herbivore was consistent with the previous studies in which belowground herbivores were distributed deep in the soil under dry conditions (Lafrance 1968; Jones 1979; Villani and Wright 1988). The belowground herbivores may graze fine roots deep in the soil, which may promotes root turnover and enhances the absorption of resources (Ramsell et al. 1993). Belowground herbivory can thus affect plant growth in either a negative or a positive way, depending on the heterogeneity of the water supply. In contrary, with the large

amount of available water regardless of supply patterns, belowground herbivory occurred in shallow soil in the free treatment due to enough soil moisture. Therefore, plant biomass was significantly lower than that in the control treatment.

Mortality of belowground herbivores varied depending on available water, which altered plant growth. When the grubs died with enough volume of available water, plant biomass was large because of the negligible loss of roots. When the available water volume was small, herbivore mortality was higher under a heterogeneous water supply than a homogeneous one, which is consistent with previous studies (Campbell 1937; Moran and Whitham 1988; Briones et al. 1997; Riis and Esbjerg 1998). However, the effects of herbivore mortality on plant biomass were not significant under a small water volume in this experiment because only few herbivores died.

To our knowledge, this is the first experiment to evaluate the simultaneous effects of available water volume, heterogeneity of water supply, and belowground herbivory on plant growth. The results are consistent with our hypothesis: the availability and heterogeneity of water supply changed the vertical distribution and mortality of belowground herbivores, and consequently plant growth. Therefore, the heterogeneity of soil water supply should be considered in root herbivory studies (Erb and Lu 2013).

Severe climatic events attributable to climate change are already having serious consequences for plants and their herbivores (Easterling et al. 2000; Staley and

Johnson 2008; Smith 2011; Reyer et al. 2013). We observed high mortality and changes in the vertical distribution of belowground herbivores under the most extreme conditions of water supply (i.e. small volume and heterogeneous supply); these consequently affected plant growth. Therefore, changes in the effects of belowground herbivores on plant growth are likely to occur under the severe weather conditions resulting from climate change.

Funding

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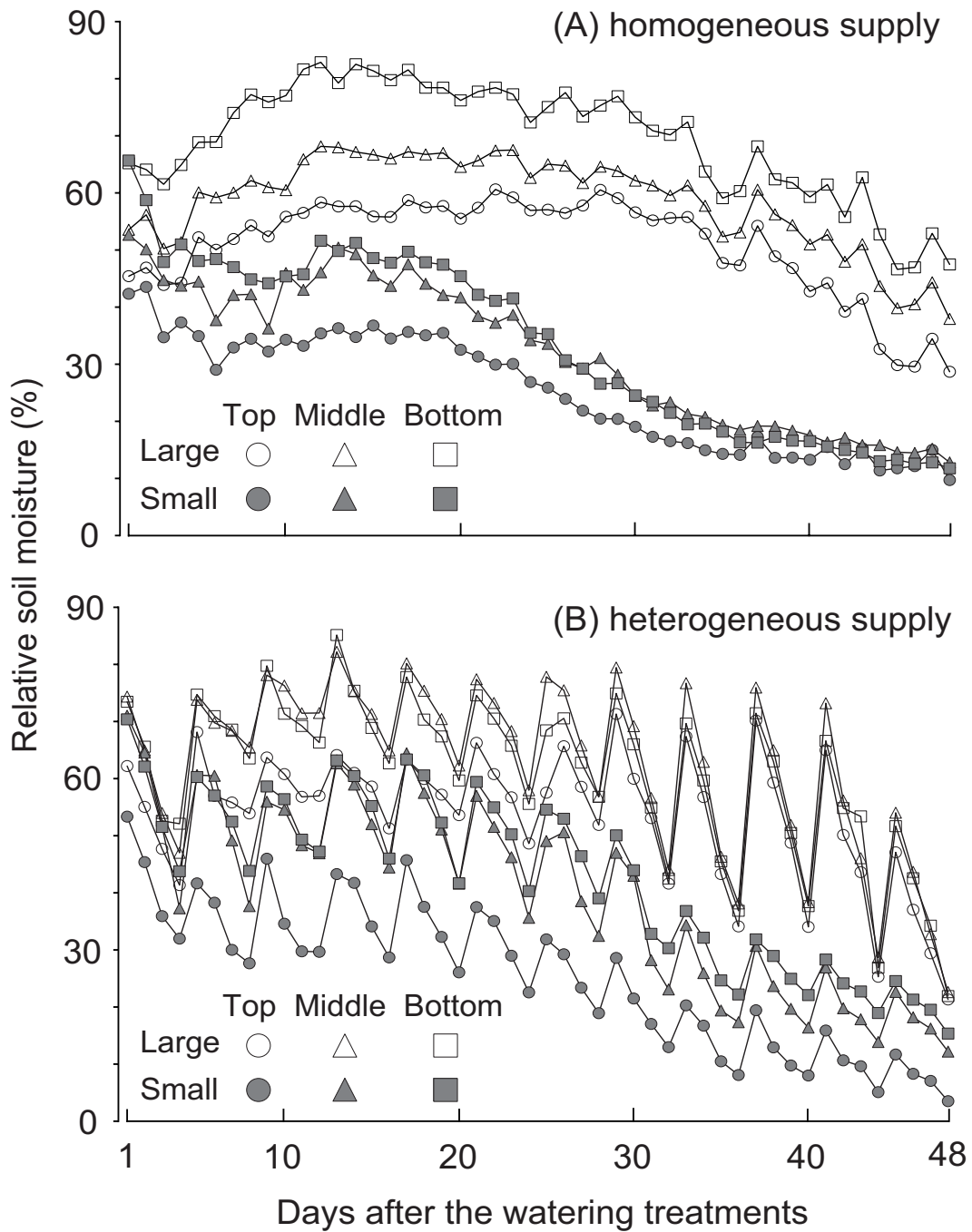


Fig. 4-1. Relative soil moisture. (A) heterogeneous supply treatments; (B) homogeneous supply treatments; Top, middle, bottom: top, middle, and bottom zone treatments, respectively. Large, large water volume supply; Small, small volume supply.

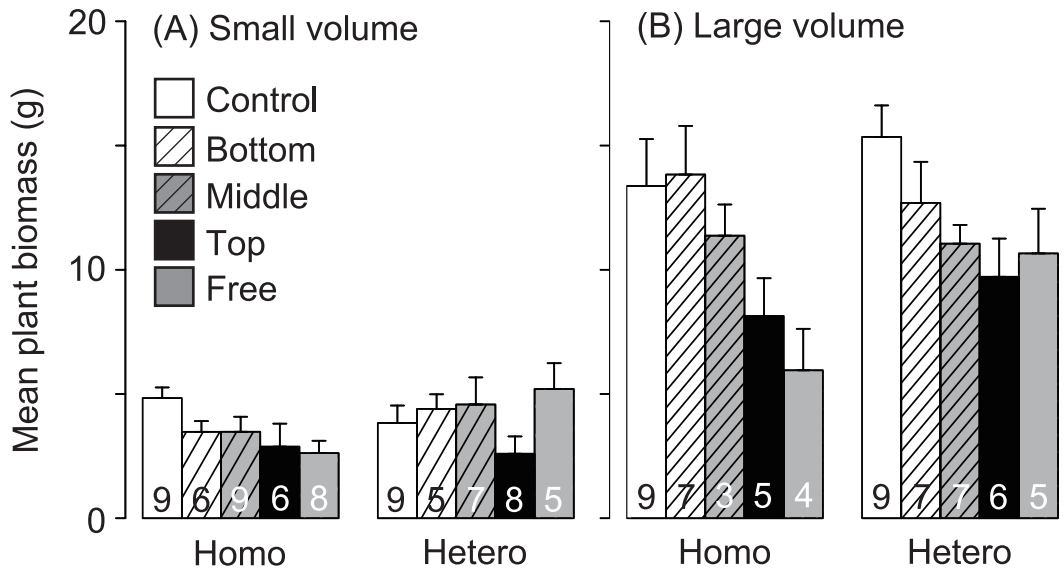


Fig. 4-2. Mean plant biomass (+ SE). (A) Small water volume; (B) Large water volume. Control, no herbivore present; Bottom, Middle, Top; herbivores placed in the bottom, middle, and top zones, respectively, of the pot; Free, herbivore present and free to move in all zones of the pot. Homo, homogeneous water supply; Hetero, heterogeneous water supply. Numbers inside each column give the sample size for each treatment combination.

Table 4-1. Plant mortality under different combinations of water volume and heterogeneity.

water volume	water heterogeneity	number of dead plants
large	homogeneous	2 (free 2)
	heterogeneous	2 (top 1, free 1)
small	homogeneous	2 (top 2)
	heterogeneous	0

Interactive effects of soil nutrient heterogeneity and belowground herbivory on the growth of plants with different root-foraging traits

Abstract

Plants with precise root-foraging patterns can proliferate roots specifically into nutrient-rich soil patches. When nutrients are distributed heterogeneously, this trait is often competitively advantageous in pot experiments but not field experiments. We hypothesized that this difference is due to belowground herbivory. We performed pot experiments using seedlings of *Lolium perenne* (a precise root-foraging species) and *Plantago lanceolata*. The experiment had a two-way factorial randomized block design, with nutrient distribution pattern (homogeneous or heterogeneous) and belowground herbivore (present or absent) as the two factors. Each pot contained one seedling of each species. With no herbivore present, plant biomass was smaller in the heterogeneous nutrient treatment than in the homogeneous treatment in *P. lanceolata*, but not in *L. perenne*. Under homogeneous nutrient distribution, in both species plant biomass was lower with a herbivore present than with no herbivore. Under heterogeneous nutrient distribution, biomass reduction due to herbivory occurred only in *L. perenne*. Roots of the precise root-foraging species were grazed more under the heterogeneous nutrient distribution, suggesting that the herbivore more efficiently foraged the roots in

nutrient-rich soil patches. The distribution and abundance of roots depending on nutrient distribution affect herbivore foraging and thus plant–plant interactions.

Keywords: Grubs, *Lolium perenne*, Nutrient patches, Plant–soil interactions, *Plantago lanceolata*, Root herbivore

Introduction

The distribution and abundance of plant resources in the environment are spatially heterogeneous at various scales (e.g., Hutchings and de Kroon 1994; James et al. 2003; Lundholm and Larson 2003), and soil nutrients, actually, scatter in soil as nutrient-rich patches (e.g., Jackson and Caldwell 1993b; Cain et al. 1999; Farley and Fitter 1999). Because species differ in root-foraging traits, they also differ in their morphological or physiological responses to nutrient-rich patches and in the magnitude of these responses (Einsmann et al. 1999; Wijesinghe et al. 2001; Rajaniemi and Reynolds 2004). Precise root-foraging species can proliferate roots specifically in nutrient-rich patches and enhance their nutrient uptake (Drew 1975; Hutchings and de Kroon 1994; Hodge 2004), whereas root-foraging species without such precise foraging do not respond to nutrient-rich patches.

Precise root-foraging species can be at an advantage in competition when nutrients are heterogeneously distributed (Hodge et al. 1999; Robinson et al. 1999; Hutchings et al. 2003), because they can capture nutrients more efficiently than species with imprecise root-foraging patterns (Hutchings and de Kroon 1994). Although many

pot experiments have shown a competitive advantage of precise root-foraging species (e.g., Robinson et al. 1999; Hodge 2003), this advantage was not evident in field studies (e.g., Bliss et al. 2002). Because pot experiments eliminate many environmental factors, the discrepancy between pot and field experiments may be attributed to unappreciated factors under natural conditions.

Belowground herbivory is often eliminated in pot experiments. However, belowground herbivores may affect plant responses to nutrient heterogeneity because they graze roots and reduce root biomass (Brown and Gange 1990; Johnson and Murray 2008). Plants are often more severely damaged by belowground herbivory than by aboveground herbivory (Blossey and Hunt-Joshi 2003; Gerber et al. 2007), with belowground herbivory sometimes causing plant mortality (e.g., Kachi 1983; Kachi and Hirose 1985; Strong et al. 1995; Coverdale et al. 2012; Tsunoda et al., in press).

Effects of belowground herbivory on species with precise root-foraging patterns, are underappreciated. Under a heterogeneous distribution of soil nutrients, precise root-foraging species proliferate their roots mainly in nutrient-rich patches, and this inevitably leads to root aggregation. Thus, nutrient-rich patches for plants can also be resource-rich patches for belowground herbivores. These patches may be preferred by belowground herbivores because they enable them to graze more efficiently (Townsend et al. 2003). Thus, compared with other plants, precise root-foraging species would suffer more from belowground herbivores under heterogeneous nutrient distributions.

The effects of belowground herbivory may differ among plant species with different root-foraging traits (e.g., Stevens et al. 2008). In a pot experiment, Stevens and Jones (2006) compared morphological and physiological responses to a heterogeneous nutrient distribution between three herbaceous species (*Andropogon ternarius* Michaux, *Eupatorium compositifolium* Walter, and *Solidago altissima* L.). When belowground herbivores (grubs, Coleoptera: Scarabaeidae) were present in pots that each contained all three species, the biomass increment of *E. compositifolium*, the most precise root forager, was significantly lower than in pots without the herbivores. However, the biomass increments of the less precise foragers did not change. Although these results suggest that the most precise forager suffered more under a heterogeneous nutrient distribution, it is possible that the herbivore species merely avoided *A. ternarius* or *S. altissima*, independent of root aggregation. Therefore, it remains to be clarified whether precise root-foraging species are more likely than other species to suffer belowground herbivory in nutrient-rich patches.

Here, we conducted a growth experiment with the precise root-foraging species *Lolium perenne* L. (Robinson and Rorison 1983; Nakamura et al. 2008) and the imprecise root-foraging species *Plantago lanceolata* L. (Maestre et al. 2005), in order to test the hypothesis that the effects of belowground herbivory differ according to root-foraging traits. We predicted that: (1) plant biomass would be smaller under a heterogeneous nutrient distribution than under a homogeneous nutrient distribution in *P. lanceolata*, but not in *L. perenne*; (2) under a homogeneous nutrient distribution, the biomass of both species would be smaller in the presence of a belowground herbivore

than without a herbivore; and (3) under a heterogeneous nutrient distribution, biomass reduction would occur mainly in *L. perenne*, because its root proliferation in nutrient-rich patches would allow for concentrated and more efficient root herbivory.

Materials and Methods

Study species

One *L. perenne* seedling and one *P. lanceolata* seedling were grown together in each pot. *L. perenne* and *P. lanceolata* commonly co-occur in seminatural temperate grasslands and pastures in which nutrient inputs are patchy and unpredictable (Grime et al. 1988; Joshi et al. 2000). The responses to soil nutrient heterogeneity differ between *L. perenne* and *P. lanceolata* (Maestre et al. 2005). Some studies have shown that the roots of *L. perenne* respond morphologically to locally supplied inorganic nitrogen (Robinson and Rorison 1983; Nakamura et al. 2008). Hodge (2003) reported that *L. perenne*, with its greater root length, captured more nitrogen than *P. lanceolata* when the two species were competing for nutrients.

For the herbivory treatment, a third-instar larva of the belowground herbivorous insect *Popillia japonica* Newman (Coleoptera: Scarabaeidae, hereafter “grub”) was added to each pot. These grubs occur in pastures and feed on various herbaceous species (Crutchfield and Potter 1994; Potter and Held 2002; Sakai and Fujioka 2007), including *L. perenne* and *P. lanceolata* (T. Tsunoda, personal observation). Grubs were grown from eggs laid in humus in the laboratory by adult *P. japonica* collected from a floodplain of the Ohta River in Tokyo (35°37'N, 139°23'E) in

summer 2009. Seeds of *P. lanceolata* were collected from the same Ohta River floodplain in summer 2009. Seeds of *L. perenne* cv. *amazing* were obtained from a commercial supplier (Snow Brand Seed Co., Ltd, Sapporo, Japan).

Experimental design

The growth experiment was conducted from December 2009 to February 2010 in a glass greenhouse under natural sunlight at the experimental garden of Tokyo Metropolitan University (Hachioji, Tokyo: 35°37'N, 139°23'E). The temperature of the greenhouse was kept over 15°C. Seeds of each species were sown on wet quartz sand in a growth chamber (Koitotron PC-02, Koito Industries, Ltd., Kanagawa, Japan) at 25°C and under a 14:10-h day:night cycle. Five-day-old seedlings were transplanted into plastic pots (14-cm diameter, 18-cm height) on 25 December 2009. One seedling of each species was placed in each pot, which was filled with 1200 ml of a mix of granular red clay and black soil (ratio 1:1 v/v). Pots were watered over the course of the study if the soil surface was dry.

The experiment had a two-way full factorial randomized block design with 16 replications; the two factors were nutrient distribution pattern and belowground herbivore. The nutrient distribution pattern treatment had two levels: homogeneous or heterogeneous distribution. The belowground herbivore treatment also had two levels: presence or absence of a grub.

Under homogeneous nutrient conditions, 4 g of slow-release fertilizer (Magamp K, 6:40:6:15 [N-P-K-Mg], Hyponex Japan, Osaka, Japan) was distributed

evenly in the soil (Fig. 5-1A). Under heterogeneous conditions, the same amount of slow-release fertilizer was also supplied, but its distribution differed; the soil of the pot was divided into quarters, and each pot consisted of a nutrient-rich quarter and three nutrient-poor quarters (Fig. 5-1B). The nutrient-rich quarter included 3 g of slow-release fertilizer, and the three nutrient-poor quarters each had 0.33 g of fertilizer. In the homogeneous treatment, the two seedlings were planted 3 cm away from the center of the pot in opposite quarters (Fig. 5-1A). In the heterogeneous treatment, the two seedlings were planted in nutrient-poor quarters on opposite sides of the pot, and the distance from each seedling to the nutrient-rich quarter was the same (Fig. 5-1B).

In the herbivore-present treatment, one grub was introduced to the pot on day 28 after transplanting. The grub was added to the center of the pot, and it burrowed under the soil surface. Plant survival was recorded every day after introduction of the grub. On day 28 after the introduction, plants were harvested and divided into shoots and roots of each species. We did not note whether roots were distributed in nutrient-rich or nutrient-poor quarters. The shoots and roots were then dried at 72°C for 3 days and weighed. Plant biomass was defined as the sum of the shoot and root biomass of each plant. At harvest, the survival of grubs was also recorded.

Statistical analyses

Total plant biomass, shoot biomass, and root biomass were each analyzed with a generalized linear mixed model (GLMM) assuming a Gaussian error distribution. In these models, the response variable was biomass and the explanatory variables were

nutrient heterogeneity, presence or absence of the belowground herbivore, plant species, and their interactions. The random factor was block. For each nutrient heterogeneity treatment, biomass was also analyzed as the response variable, and the explanatory variables were plant species, presence or absence of the belowground herbivore, and their interactions.

In the plant biomass analyses, if the assumption of homogeneity of variance was satisfied according to Bartlett's test, then an identity-link function was applied. If not, then a log-link function was applied. Those pots in which belowground herbivores or plants died were treated as missing values.

All analyses were performed with the statistical software R version 2.15.1 (R Development Core Team 2012). The lme4 package was used to calculate GLMMs by using maximum likelihood estimation. To determine the effects of the fixed factors, we used Wald tests and the chi-squared test statistic.

Results

Two *L. perenne* plants and one *P. lanceolata* died in the heterogeneous nutrient distribution treatment with a grub. These dead plants had been grazed at the root base, and their shoots were severed from the roots. No plant died in the homogeneous nutrient distribution treatment or in the treatment without a grub. Two grubs died in the homogeneous nutrient distribution treatment, and three grubs died in the heterogeneous treatment. Pots in which the mortality of a plant or a grub was observed were excluded from the following analyses.

Mean plant biomass was significantly affected by the three-way interaction among nutrient heterogeneity, belowground herbivore, and plant species ($\chi^2 = 5.687$, $df = 1$, $P = 0.017$), which together influenced plant growth. Means of shoot and root biomass were also significantly affected by this three-way interaction (mean shoot biomass: $\chi^2 = 4.835$, $df = 1$, $P = 0.028$; mean root biomass: $\chi^2 = 5.487$, $df = 1$, $P = 0.019$).

When no belowground herbivore was present (Fig. 5-2A), the effects of nutrient heterogeneity on plant growth differed between plant species (two-way interaction between nutrient heterogeneity and plant species: $\chi^2 = 7.288$, $df = 1$, $P = 0.007$). The mean plant biomass of *L. perenne* did not differ between the homogeneous and heterogeneous nutrient distributions, whereas the total biomass of *P. lanceolata* was smaller in the heterogeneous treatment. The effects of nutrient heterogeneity on shoot and root growth also differed between plant species (two-way interaction between nutrient heterogeneity and plant species, mean shoot biomass: $\chi^2 = 5.400$, $df = 1$, $P = 0.020$; mean root biomass: $\chi^2 = 13.608$, $df = 1$, $P < 0.001$). The mean shoot and root biomasses of *L. perenne* did not differ between nutrient treatments, whereas those of *P. lanceolata* were smaller under the heterogeneous nutrient distribution. Under the homogeneous treatment, root biomasses of the two species were similar. However, in the heterogeneous treatment, the root biomass of *L. perenne* was twice as large as that of *P. lanceolata*.

Under the homogeneous nutrient distribution (Fig. 5-2B), mean plant biomass significantly decreased in *L. perenne* and *P. lanceolata* when a belowground herbivore

was present ($\chi^2 = 4.706$, $df = 1$, $P = 0.030$). With a herbivore present, mean root biomass also significantly decreased in both species ($\chi^2 = 6.139$, $df = 1$, $P = 0.013$), and the decreases in mean shoot biomass were marginally significant ($\chi^2 = 3.604$, $df = 1$, $P = 0.058$).

Under the heterogeneous nutrient distribution (Fig. 5-2C), the effect of belowground herbivory on mean plant biomass differed between plant species (two-way interaction between herbivory and plant species, $\chi^2 = 9.196$, $df = 1$, $P = 0.002$). When a belowground herbivore was present, the mean plant biomass of *L. perenne* decreased, but that of *P. lanceolata* did not change. The effect of belowground herbivory on mean shoot and root biomasses also differed between plant species (shoot mass: $\chi^2 = 5.877$, $df = 1$, $P = 0.015$; root mass: $\chi^2 = 16.377$, $df = 1$, $P < 0.001$). When a belowground herbivore was present, the mean shoot and root biomasses of *L. perenne* decreased, whereas those of *P. lanceolata* did not change.

Discussion

Plant mortality occurred only under heterogeneous nutrient distribution in the presence of a grub. The mortality of *L. perenne* was attributed to the intensive herbivory, and that of *P. lanceolata* to small plant size.

When no grub was present, the biomass of *P. lanceolata* was smaller under the heterogeneous nutrient condition, whereas *L. perenne* biomass did not differ significantly between the two nutrient distribution patterns. In line with our first prediction, the results revealed differences in the responses to nutrient heterogeneity

between *P. lanceolata* and *L. perenne*. The lack of a significant difference in biomass of *L. perenne* between the homogeneous and heterogeneous nutrient treatments was attributed to those precise root responses to nutrient heterogeneity, which were indicated by Robinson and Rorison (1983) and Nakamura et al. (2008). In contrast, the small biomass of *P. lanceolata* under heterogeneous conditions suggests imprecise foraging of nutrient patches (see also Maestre et al. 2005). The difference in root-foraging traits between these species is consistent with the findings of Hodge (2003), who showed that *P. lanceolata* is competitively inferior to *L. perenne* under a heterogeneous nutrient distribution.

Under homogeneous conditions, the smaller biomass of *P. lanceolata* and *L. perenne* in the presence of a grub strongly suggests that both species suffered from belowground herbivory, which is consistent with our second prediction. The decreases in biomass of both species can be attributed to root herbivory, because *P. japonica* grubs are generalist feeders (Crutchfield and Potter 1994; Potter and Held 2002; Sakai and Fujioka 2007). There would be no difference in the grub's grazing efficiency between the two plant species under homogeneous conditions, because no root aggregation would occur in this case. In fact, there was no significant difference in root biomass between *L. perenne* and *P. lanceolata* (Fig. 5-2B). Therefore, roots were grazed to a similar degree in both species.

Under heterogeneous conditions, the root biomass of *L. perenne* was significantly smaller when a grub was present than when one was absent; this is consistent with our third prediction. Previous studies have shown that *L. perenne*

forages in nutrient-rich patches under heterogeneous nutrient conditions and enhances its growth (e.g. Robinson and Rorison 1983; Nakamura et al. 2008). In fact, under heterogeneous conditions without a grub, the root biomass of *L. perenne* was twice as large as that of *P. lanceolata* (Fig. 5-2C). Thus, the grub could efficiently feed on the aggregated roots of *L. perenne*. When a grub was present under these nutrient conditions, *P. lanceolata* gained more biomass than *L. perenne*, indicating that the presence of a belowground herbivore altered the hierarchy in interspecific competition.

As we hypothesized, the effects of belowground herbivory on plants differed between plant species with different root-foraging traits. Stevens and Jones (2006) also reported various effects of belowground herbivory on plants with different foraging traits. In their experimental design, however, it was possible that herbivores grazed only on certain species. Our results strongly indicated that the belowground herbivore grazed on both species. These findings show that the distribution and abundance of roots, which depend on the nutrient distribution, affect the foraging behavior of herbivores and thus their choice of food.

Our experiment revealed that the interactive effects of nutrient heterogeneity and belowground herbivory on plants are crucially important, because the fate of plants is often determined by belowground herbivory (Kachi 1983; Kachi and Hirose 1985; Strong et al. 1995; Blossey and Hunt-Joshi 2003; Coverdale et al. 2012; Tsunoda et al., submitted). Our results suggest that plant growth and mortality are altered by the interaction between nutrient heterogeneity and belowground herbivory. Such interactions affect competitive relationships between plants and thus the coexistence of

plant species, ultimately exerting deterministic influences on a community and its productivity (de Kroon et al. 2012).

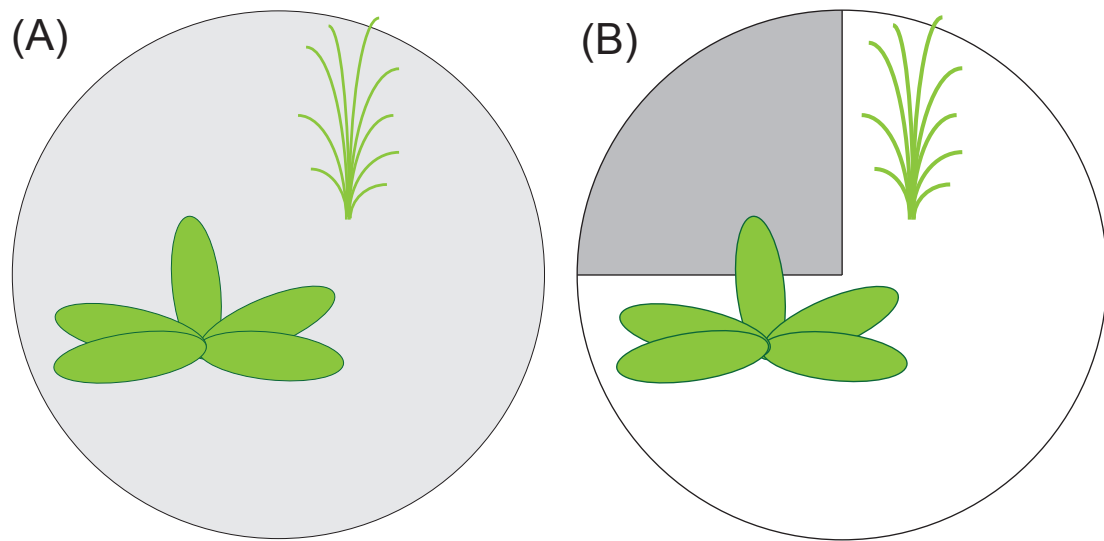


Fig. 5-1. In each pot, *Lolium perenne* and *Plantago lanceolata* seedlings were planted 6 cm apart. (A) Under homogeneous nutrient conditions, 4 g of slow-release fertilizer was distributed evenly in the soil. (B) Under heterogeneous conditions, the nutrient-rich quarter (gray) received 3 g of slow-release fertilizer and each of the three nutrient-poor quarters received 0.33 g of fertilizer.

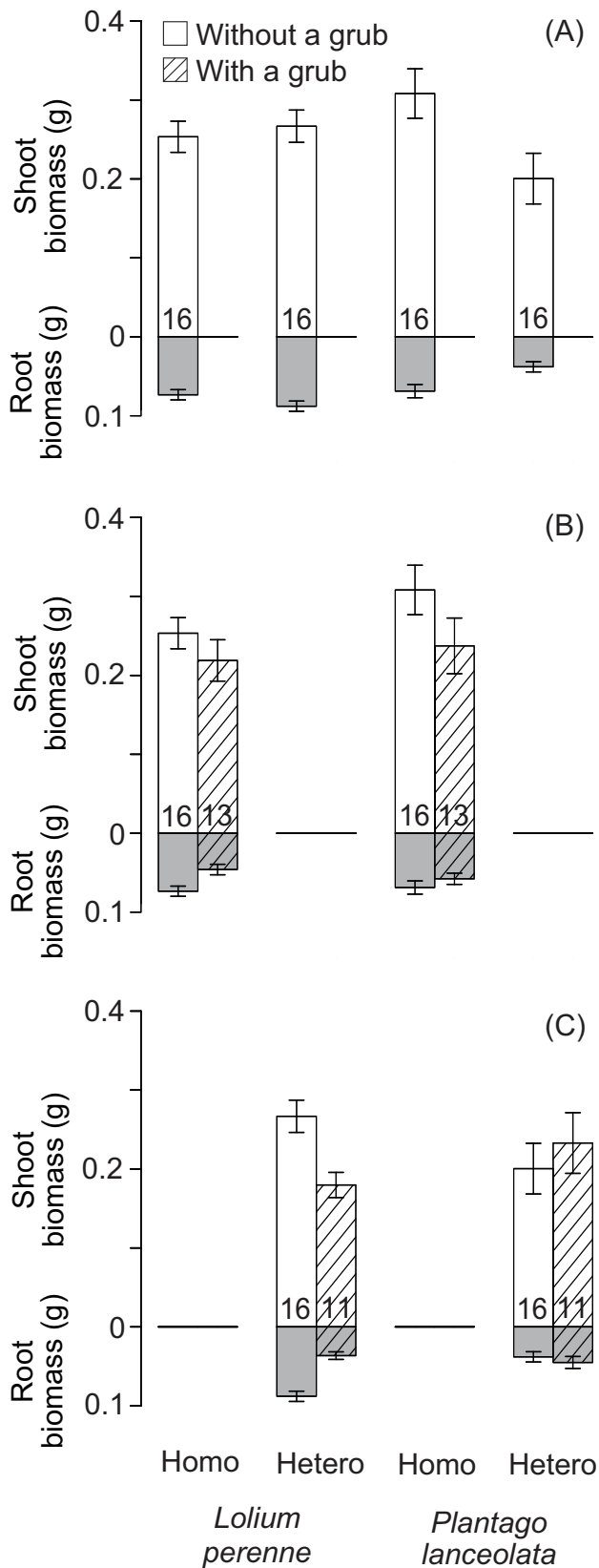


Fig. 5-2. Shoot (open bars) and root (gray bars) mean biomass (\pm SE). Treatments with (A) no grub present; (B) a homogeneous nutrient distribution; and (C) a heterogeneous nutrient distribution. Numbers inside each column give the sample size for each treatment combination.

Chapter VI

General Discussion

This thesis demonstrated that the effects of belowground herbivory on plant growth and survival differed depending on the vertical distribution of a belowground herbivore, plant growth stages, root architecture, and the belowground heterogeneity of water and nutrients. In this chapter, I discuss the growth and survival of plants from belowground herbivory, the ecology of scarabaeid larvae and plant defense against belowground herbivory.

The vertical distribution of a belowground herbivore

The vertical distribution of a belowground herbivore is crucial for the growth and survival of plants if the herbivores did not differ in species and density (Chapter 2, Fig. 6-1). Belowground herbivore species (Steinger and Müller-Schärer 1992; Wurst and van der Putten 2007; Erwin et al. 2013) and density (Masters 1995; Strong et al. 1995; Onstad et al. 2006; Simelane 2010) are known to affect the extent of belowground herbivory on plants. However, to my knowledge, Davidson and Roberts (1969) are the only authors discussing the effects of vertical distribution of belowground herbivores on plants.

The vertical distribution of belowground herbivores depends on soil conditions. Indeed, drying soil drives belowground herbivores deeper into the soil profile (Lafrance 1968; Jones 1979; Villani and Wright 1988). The descent of *Anomala cuprea* larvae in the soil profile was attributed to drought in the surface soil from the small heterogeneous supply of water (Chapter 4). The results of my thesis suggest that soil water status often depended on patterns of water supply. In fields, precipitation patterns that determine the soil water status could alter the vertical distribution of belowground herbivores and their effects on plants.

Plant growth stages and root architecture

The effects of belowground herbivory on plant growth and survival depended on several root system traits associated with the growth stage (Chapter 3, Fig. 6-2). At early growth stages when the taproot of *Plantago lanceolata* was thin, the relative small amount of roots was followed by a reduction in plant biomass and survival from belowground herbivory (Fig. 6-2A). At late growth stages, the amount of root was relatively large and *P. lanceolata* had developed a taproot, with no reduction in plant biomass and survival (Fig. 6-2B). Root architecture and abundance could explain why the effects of belowground herbivory on plants are different between plant species or plant functional groups (Zvereva and Kozlov 2012). Plant defenses, which mediate belowground plant-herbivore interactions (Bezemer and van Dam 2005; Rasmann and

Agrawal 2008; van Dam 2009), differ depending on the growth stage of the plant (e.g., Boege and Marquis 2005; Quintero and Bowers 2012). Therefore, root defense should be evaluated at each growth stage in future studies.

The diverse response of plant communities to belowground herbivory can be attributed to the variable response of individual plant species to herbivory depending on the growth stage and root architecture. Belowground herbivory has different effects on species diversity in plant communities because of their successional stages (Brown and Gange 1989, 1992; Stein et al. 2010). In an early-successional community with low productivity, belowground herbivores reduced forb abundance relative to the grasses, decreasing plant species diversity and encouraging successional transition (Brown and Gange 1989, 1992). Forb seedlings showed more detrimental effects from belowground herbivory compared with grass species (Chapter 3). Thus, under a certain level of belowground herbivory, forbs would be less likely to establish in a community at an early successional stage. In a community dominated by grass species, plant species richness increased because of belowground herbivory affecting grasses more compared with forbs (Stein et al. 2010), probably because herbivory formed gaps in which non-dominant species could establish.

The feeding sites of belowground herbivores

The feeding sites of belowground herbivores require further investigation (i.e., taproots,

lateral roots, fine roots, and rhizomes), because feeding sites may determine herbivory damage (Schoonhoven et al. 2005; Chapter 2; Chapter 3). Aboveground herbivory is categorized such as folivory, florivory, or seed predation depending on the feeding site, whereas belowground herbivory is not categorized because it is rarely observed. However, some feeding strategies such as chewing, sucking and boring, are known to exist (Brown and Gange 1990; Zvereva and Kozlov 2012), suggesting the existence of different root feeding sites. The diversity of feeding sites on root systems may have a varied effect on plants.

Heterogeneity of water and nutrients in soil

The heterogeneity of the water supply and soil nutrient distribution affects belowground herbivory and plant growth, even when similar amounts of water and nutrients were provided (Chapter 4, Fig. 6-3; Chapter 5, Fig. 6-4). The damage caused by belowground herbivory was enhanced under low soil moisture regimes (Blossey and Hunt-Joshi 2003; Zvereva and Kozlov 2012). However, my results that root herbivory was mitigated under low water supply could be contentious. Drought in shallow soils from a heterogeneous water supply caused belowground herbivores to migrate deeper into the soil profile (Fig. 6-3A). Thus, shallow roots that were crucial in absorbing soil resources were less likely to incur herbivory damage. Research demonstrating the effects of multiple resource supply on herbivory appears controversial even in aboveground parts

(Hawkes and Sullivan 2001; Wise and Abrahamson 2005, 2007; Banta et al. 2010; Bagchi and Ritchie 2011). Further research is required to elucidate the mechanisms of how the heterogeneous combination of water and nutrients in the soil affects above- and belowground herbivory.

The ecology of scarabaeid larvae as belowground herbivores

Understanding the specific response of belowground herbivores to the soil environment is critical (e.g., Staley et al. 2007). Scarabaeidae is the largest taxa of generalist root-chewers (Whittaker 2003; Zvereva and Kozlov 2012), with *Popillia japonica* one of most well researched species (e.g., reviewed by Fleming 1972; Potter and Held 2002). On this basis, I used the generalist root-chewing larvae of *A. cuprea* and *P. japonica* (Coleoptera: Scarabaeidae) in my experiments.

A. cuprea showed high mortality under dry conditions (Chapter 4) when associated with *P. japonica* (Régnière et al. 1981) and other belowground herbivores (Campbell 1937; Moran and Whitham 1988; Briones et al. 1997; Riis and Esbjerg 1998). Under dry soil conditions soil dwelling arthropods descend further into soil profile, as shown by *P. japonica* (Villani and Wright 1988), carrot-fly larva *Psila rosae* (Jones 1979) and wireworms (Lafrance 1968). Many belowground herbivore species belonging to various taxa are considered susceptible to soil moisture especially under dry conditions (Brown and Gange 1990; Barnett and Johnson 2013).

The larvae of *A. cuprea* are normally found in shallower areas of the soil profile (Chapter 2). Consistent with my findings (Chapter 2), over 90% of *P. japonica* larvae occurred in the first 50 mm of the soil profile during their growth period, as *P. japonica* larvae sever the roots from the plant during feeding resulting in the death of the plant (Fleming 1972). Alternatively, *P. japonica* migration deeper into the soil profile could be in response to drought (Villani and Wright 1988) or seasonal conditions (Fleming 1972; Potter and Held 2002).

Scarabaeid larvae are known to kill their host plants (e.g., Fleming 1972; Kachi 1983; Potter and Braman 1991), which would inevitably reduce their diets owing to the death of plants. However, as generalists, scarabaeid larvae can forage for alternative hosts.

Under field conditions, identifying the diet of generalist belowground herbivores is difficult because the herbivore can choose its host from various species. However, stable isotope analysis (Schallhart et al. 2011) and DNA barcoding (Staudacher et al. 2011; Wallinger et al. 2013) can address this issue, as studies on the feeding behavior of wireworms in agroecosystems have shown (e.g., Staudacher et al. 2013). These techniques are applicable to herbivorous soil fauna affecting plant growth and their interactions even under field conditions.

In belowground ecosystems, there are various specialist herbivores, such as cabbage root maggot, *Delia radicum* (Diptera: Anthomyiidae), and western corn rootworm, *Diabrotica virgifera* (Coleoptera: Chrysomelidae), although specialist herbivores are less abundant in grasslands. The feeding behavior of specialists often

differs from that of generalists (e.g., Robert et al. 2012) because some specialist herbivores exhibit tolerance against the defense compounds of plants, which are actually cues for some specialists to forage their hosts (Hopkins et al. 2009). Thus, defensive compounds would determine feeding sites of belowground herbivores. Because feeding sites determine plant damages from belowground herbivory (Chapter 2, Chapter 3), feeding sites of specialist herbivores require further investigation.

The defense strategy of a root system: allocation and timing

The optimal defense hypothesis (ODH) is one of the most influential hypotheses, describing plant defense both qualitatively and quantitatively (McKey 1974; Zangerl and Bazzaz 1992; Meldau et al. 2012). The main premise of the ODH is that defense compounds should be allocated to those parts that would be most effective to enhance plant fitness (e.g., young leaves or reproductive organs), because there are limited plant resources to produce defensive metabolites.

Few studies have tested the ODH on belowground plant organs. The ODH predicts that defensive investment should be largest in upper root system (van Dam 2009). Root herbivory was most destructive to plants near the soil surface (Chapter 2), suggesting that the concentration of defensive compounds should be greatest in the upper root system according to the ODH. The defensive values of top roots should be different depending on growth stages (Chapter 3). For example, the concentration of

defensive compounds in *P. lanceolata* roots was higher in 18-week-old plants compared with younger plants, but the study did not distinguish root sites (Quintero and Bowers 2012). The possibility exists that the taproots are grazed differently depending on the plant growth stage. Therefore, belowground plant defense for each root site and plant growth stage requires further evaluation.

Concluding remarks

Belowground herbivores and plant roots exist in a dynamic and heterogeneous environment (de Kroon et al. 2012; Erb and Lu 2013). To understand the various effects of belowground herbivory on plants, soil heterogeneity requires experimental investigation.

In this thesis, I demonstrated that plant growth and survival depended on the interaction between belowground herbivory and the heterogeneity of soil water and nutrients. Belowground herbivores and the heterogeneity of water and nutrients in soil are known as determinants of plant productivity and species coexistence in plant communities (Grime 1979; Tilman 1988; Hutchings and de Kroon 1994; Townsend et al. 2003; Hodge 2004; Bardgett 2005; de Kroon et al. 2012). The effects of the factors investigated on the experimental plants was not additive. Therefore, it is important to consider species traits when evaluating the interactive effects on plant productivity and species coexistence in plant communities.

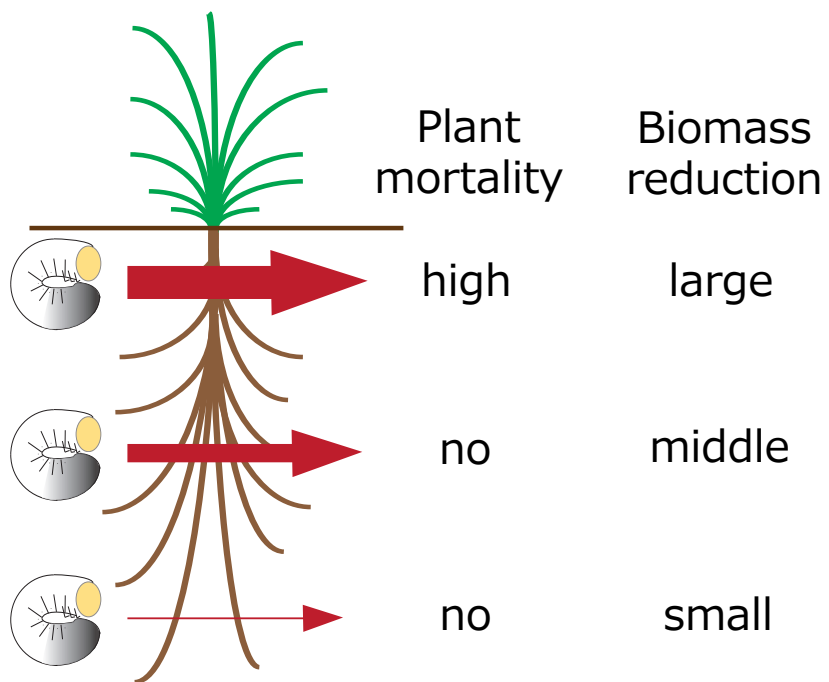


Fig. 6-1. Conceptual diagram of the effects of belowground vertical distribution of a herbivore.

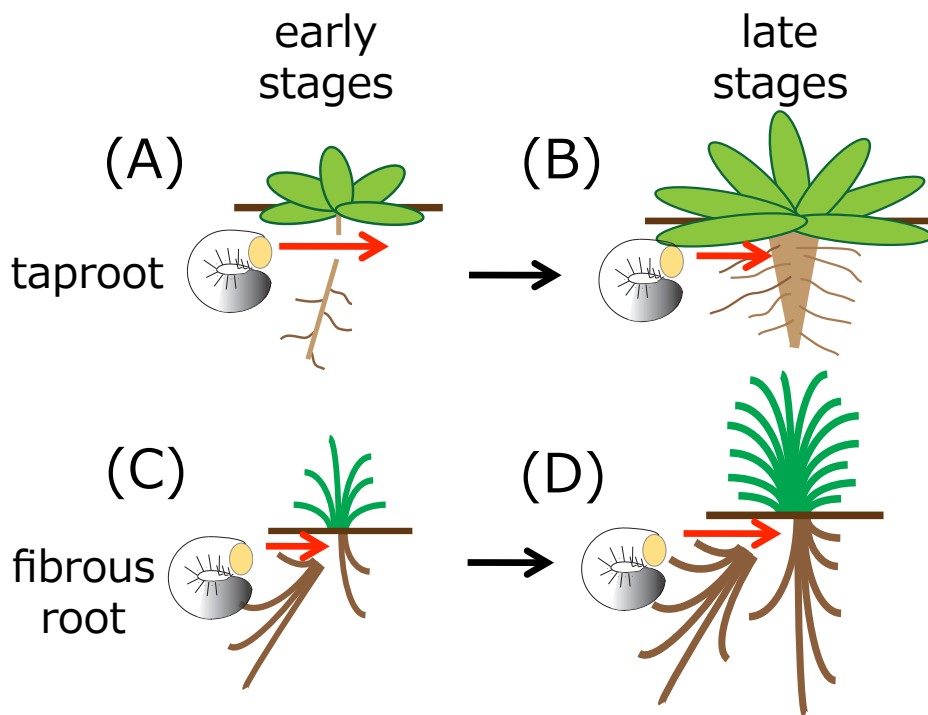
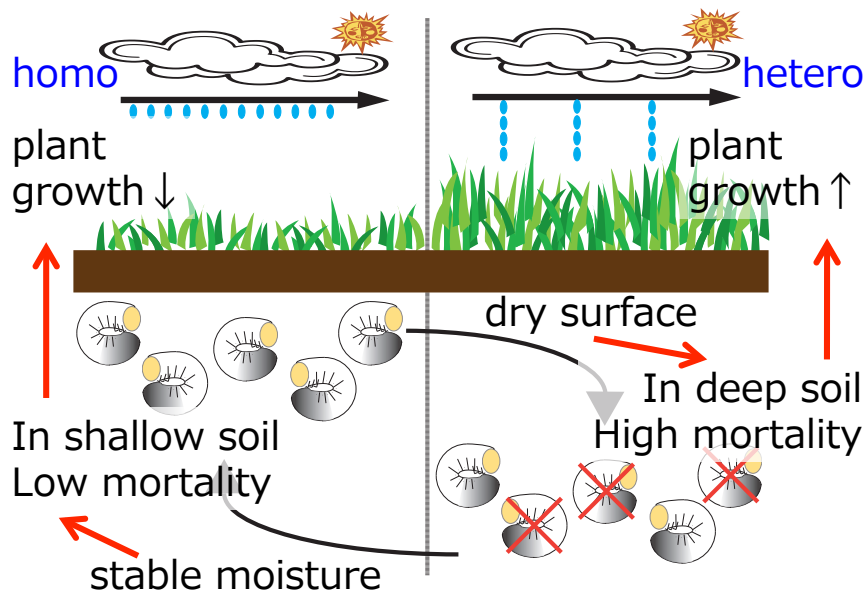
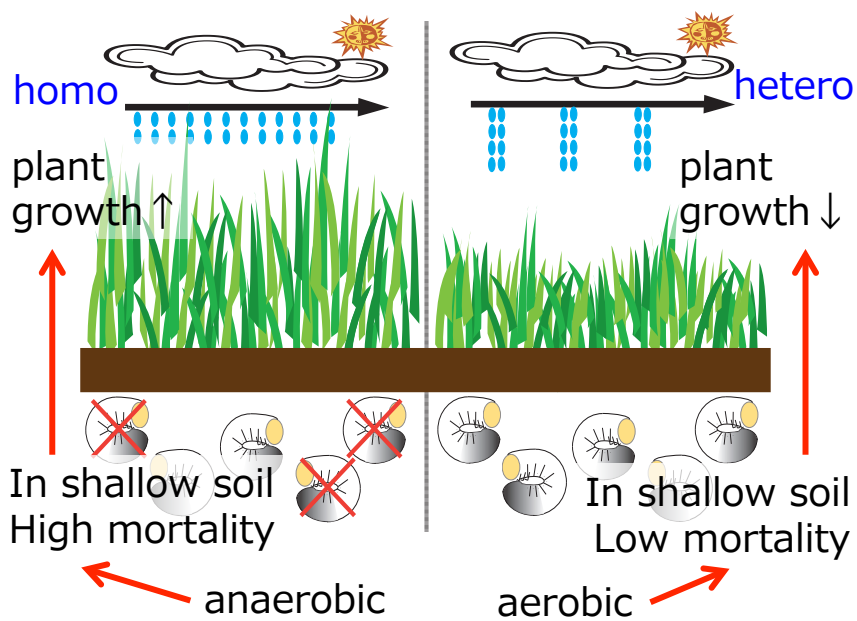


Fig. 6-2. Conceptual diagrams of the effects of belowground herbivory on each growth stage of *Lolium perenne* and *Plantago lanceolata*. (A) Taproot system of *P. lanceolata* at early growth stages loses almost all roots by belowground herbivory; (B) Taproot system of *P. lanceolata* at late growth stages is not affected by belowground herbivory; (C) Fibrous root system of *L. perenne* at early growth stages loses small amounts of roots by belowground herbivory relative to *P. lanceolata*; (D) Fibrous root system of *L. perenne* loses their roots even at late growth stages.



(A)



(B)

Fig. 6-3. Conceptual diagrams of the effects of availability and temporal heterogeneity of water supply on soil conditions, belowground herbivores, and plant growth. (A) Small water volume; (B) Large water volume.

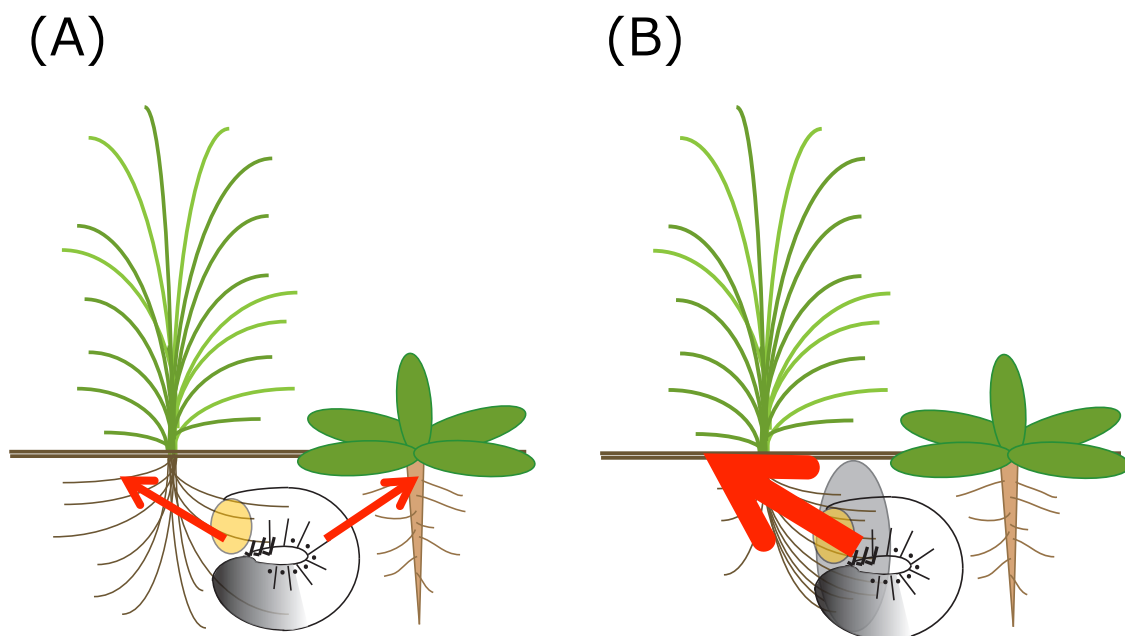


Fig. 6-4. Conceptual diagrams of the plant selection by a larva of *Popillia japonica* under the different nutrient distributions in soil (A) Under homogeneous nutrient distribution, the larva feed upon roots randomly; (B) Under heterogeneous nutrient distribution, the larva select and feed upon the roots of precise root-foraging species.

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植物の生残と成長におよぼす地下部の根食と
環境不均質性の影響の実験生態学的研究（英文）

生物が成育する環境は、時間的・空間的に不均質である。土壤環境においても、さまざまなスケールで土壤水分や栄養は不均質である。それに応答して、植物の根系や土壤動物は分布や量を変化させる。植物と土壤動物の分布や量が異なると、植物への土壤動物の影響やその強さは変化する。従って、土壤環境の不均質性に起因する植物と土壤動物の分布と量の変化により、植物への土壤動物の影響が変化する可能性がある。

植物の生残や成長および個体間相互作用は、被食により影響される。地下部での被食（根食）は、地上部での被食より影響が大きい。また、植物と根食昆虫（根食者）の挙動は、土壤水分や栄養の総量により変化する。土壤水分や栄養の総量が異なれば根食が植物に及ぼす影響も変化する。一般に、湿度が低く貧栄養な環境だと、根食の影響をより強く植物は受ける。一方、土壤水分や栄養の総量の影響を受けた根食者が、植物に及ぼす影響が変化するかの研究例は少ない。また、土壤水分や栄養の時間的・空間的な不均質性が、植物と根食者の挙動を変化させ、根食が植物におよぼす影響を変化させるかは検討されてこなかった。

そこで、本研究は、栽培実験により、植物の生残・成長と種間相互作用に根食が及ぼす影響を、根食者の垂直分布と植物根系の特性に注目し、評価した。

さらに、根食者の垂直分布と根の空間分布を変化させる環境不均質性が根食の影響をどのように変化させるかも評価した。植物材料にはホソムギ *Lolium perenne* L.とヘラオオバコ *Plantago lanceolata* L.を用いた。また、根食者としては、野外で優占する広食性根食者、コガネムシ科 *Scarabaeidae* 幼虫を用いた。

まず、植物の生残と成長が、根食者の垂直分布により変化することを明らかにした。一個体のホソムギを鉢で一定期間栽培し、一匹の根食者 *Anomala cuprea* Hope を加え、さらに生育した後、刈取り、ホソムギの枯死数と乾燥重量を求めた。根食者については、5条件（根食者がいる層が土壌の上層、中層、下層、移動制限無し、根食者無し）を設定した。ホソムギが生育する土壌中で根食者の垂直分布を限定した場合、根食者の分布が上層のみだと、多くのホソムギは枯死し、生残したホソムギの個体重も極めて小さかった。下層のみに分布した場合の個体重は大きく、枯死も見られなかった。ホソムギの枯死は根食者の移動に制限がない場合にも見られた。根食者は浅い土壌に多く分布し、植物は根元を根食されると考えられた。

次に、根系の形態が異なる2種と生育段階によって、植物の生残と成長に根食が及ぼす影響の変化を明らかにした。植物の種（ホソムギかヘラオオバコ）と生育段階（植物のみでの栽培日数が15日か25、35、45日）、根食者 *A. cuprea*（有か無）を3要因とし、鉢で育成した。一個体のホソムギかヘラオオバコを鉢で一定期間栽培し、一匹の根食者を加え、さらに生育した後、刈取り、植物の枯死数と乾燥重量を求めた。植物のみでの栽培日数が短い、小さい生育段階ほど、根食により枯死する植物がどちらの種でも多かった。主根状のヘラオオバコで、ひげ根状のホソムギに比べ、より顕著な枯死が見られた。生育段階に

関わらず生残したホソムギでは、根食により個体重が減少した。ヘラオオバコでは根食による個体重の変化が、生育後期には見られなかった。

さらに、植物に対する根食の影響が、給水総量と給水様式の相互作用により変化することを明らかにした。給水総量（多か少）と給水様式（均質（ある量を毎日給水）か不均質（その4倍量を4日に一度給水））の組み合わせのもと、一個体のヘラオオバコを鉢で一定期間栽培し、一匹の根食者 *A. cuprea* を加え、さらに育成した後、刈取り、ヘラオオバコの乾燥重量を求めた。根食者については、5条件（根食者がいる層が土壌の上層、中層、下層、移動制限無し、根食者無し）を設定した。給水総量が多い条件の土壌と、給水の総量や様式を問わず土壌の下層では、湿度が高かった。土壌湿度の変動は、均質より不均質な給水様式で大きかった。給水総量が少なく根食者がいる場合、均質な給水様式だとヘラオオバコの個体重は減少し、不均質な給水様式だと個体重は増加した。これは、土壌湿度に応答し、根食者の土壌中での垂直分布が変化したためだと考えられた。給水総量が多いと、給水様式に関わらずヘラオオバコの個体重は減少した。これは、根食者が土壌上層に分布し、根食により多くの根が失われた結果だと考えられた。また、根食者の死亡が多かったのは、給水総量が多いときは均質な給水様式、給水総量が少ない時は不均質な給水様式だった。給水総量が多い条件では、根食者が死亡していたヘラオオバコの個体重が生残していたものより大きかった。

最後に、土壌栄養塩分布の不均質性に応じて、根食者が植物に及ぼす影響が変化し、植物の種間相互作用に影響することを明らかにした。同所的に生育するホソムギとヘラオオバコでは、局所分布する土壌栄養塩への応答性が異なる。

異なる土壤栄養塩分布（均質か不均質）のもと、両種を一個体ずつ同一の鉢で一定期間栽培し、一匹の根食者 *Popillia japonica* Newman を加え、さらに育成した後、刈取り、植物の乾燥重量を求めた。土壤栄養塩が不均質に分布する場合、栄養塩への応答性の高いホソムギで、個体重と地下部重量が大きかった。しかし、同じ量の土壤栄養塩が均質に分布すると、ホソムギとヘラオオバコの間で、個体重および地下部重量に差はなかった。栄養塩分布が均質な場合、根食者の存在により、両種の個体重がともに減少した。栄養塩分布が不均質な場合、根食者の存在により、ホソムギのみ個体重が減少した。根食者が摂食したのは、均質な分布では両種、不均質な分布では主にホソムギだと考えられた。

植物が受ける根食の影響が変化したのは、根食者の垂直分布や植物の生育段階および根の形態的特性によって、根食により失われる根の量が異なったためだと考えられる。植物の生育段階が小さい場合や土壌の上層に根食者が分布する場合に、多くの植物で、ほぼ全ての根が失われ、枯死が見られた。野外では、根食者は地表付近に多く分布するので、根食者は実生の加入を減少させたり、影響の受けやすい種の現存量を選択的に減少させたりしていると考えられる。

環境の不均質性により、植物と根食者は変化し、根食の影響も変化した。不均質な給水により、土壌湿度が上層で低くなると、根食者は下層へ移動し、植物の成長への影響が小さくなったと考えられる。湿度が低く根食者がいると、根食の影響はより強く植物に現れるとこれまでは考えられていた。しかし、不均質な土壌湿度に根食者も応答し、湿度が低い浅い土壌から、湿度が高い深い土壌に移動したと考えられ、植物への影響が変化することが明らかとなった。また、土壤栄養塩分布の不均質性により、根の分布や量の応答は変化し、根食

者の作用も変化した。野外で一般的な土壌環境の不均質性が、植物と根食者双方に与える影響を検討し、植物への根食の影響を評価する必要性が示された。