

Effects of plant identity and diversity on the dietary choice of a soil-living insect herbivore

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Abstract. Plant identity and diversity influence herbivore communities in many different ways. While it is well known how they affect the feeding preferences of aboveground herbivores, this information is lacking for soil ecosystems, where examining plant–herbivore trophic interactions is difficult. We performed a mesocosm experiment assessing how plant identity and diversity affect the food choice of *Agriotes* larvae, which are soil-living generalist herbivores. We offered four plant species, (maize, a grass, a legume, and a forb) at varying combinations and diversity levels to these larvae, and analyzed their feeding behavior using stable isotopes. We hypothesized that (1) their food choice is driven by preference for certain plant species rather than by root abundance and that (2) the preference for specific plants changes with increasing plant diversity. We found that larvae preferred the grass and legume but avoided maize and the forb. Whether a plant was preferred or avoided was independent of diversity, but the extent of avoidance or preference changed with increasing plant diversity. Our findings reveal that the dietary choice of soil-living generalist herbivores is determined by plant-specific traits rather than root abundance. Our data also suggest that soil herbivore feeding preferences are modulated by plant diversity.

Key words: ¹³C; ¹⁵N; *Agriotes*; food preference; isotopic labeling; root herbivory; wireworms.

INTRODUCTION

Herbivores play a key role in most terrestrial ecosystems. They often regulate the species composition of plant communities, the total amount of plant biomass in the ecosystems, and the magnitude of ecosystem functional processes such as primary production, decomposition, and trophic transfer efficiency (Crowley 1983). Conversely, plant functional identity and diversity have been shown to drive herbivore community composition (Siemann et al. 1998, Otway et al. 2005, Scherber et al. 2006, Unsicker et al. 2006) and the population ecology of individual herbivore species (Specht et al. 2008). Despite a comprehensive body of literature concerning aboveground ecosystems, little is yet known about how plant identity and diversity affect herbivore dietary choice belowground, where a considerable part of plants' biomass is located. Unraveling these belowground effects is a prerequisite for a better understanding of the relationships between herbivore and plant communities.

Generalist herbivores can feed on multiple plant species either unselectively or selectively. In the first case, food choice depends on plant abundance, i.e., plants that produce the greatest biomass of palatable

tissues account for the highest proportion in the herbivores' diet. In the second case, food choice depends on plant-specific traits, leading to a preference for specific plant species. Such traits include species-specific nutrient composition (Behmer 2009) and nitrogen content (Mattson 1980), as well as plant defense via chemical (Kessler and Baldwin 2002) and mechanical deterrents. Although many studies have examined the influence of plant-specific traits on generalist herbivores (e.g., Bernays et al. 1994, Bernays and Minkenberg 1997, Behmer 2009), the focus has largely been on aboveground systems.

Soil systems are important habitats for herbivores because frequently more than 50% of net primary production is allocated to belowground plant parts (Coleman 1976). Furthermore, belowground species diversity is generally much higher than in the corresponding aboveground systems (Wardle 2006). Accordingly, soil ecosystems can host a variety of soil insect herbivores that can have dramatic and long-lasting effects on both natural and managed plant communities (Hunter 2001).

The living conditions of soil insect herbivores differ greatly from those of their aboveground counterparts. The substrate impedes large-scale movement and perception of plant signals, thus hampering foraging for specific plant species. Herbivores in agricultural systems also face rapid and dramatic changes in plant composition and habitat conditions due to crop rotation or cultivation practices, which pose difficulties especially to the less mobile soil herbivores. Because of these

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challenges, it is questionable whether the mechanisms that drive herbivore food choice aboveground are the same in the soil.

Examining belowground herbivory is difficult because the trophic interactions are hidden in the soil, impeding direct observation. Moreover, the interwoven roots of different plant species are hard to distinguish from each other, and species assignment of plant remains in herbivore guts or feces is even more difficult. As a result, the effect of plant community composition on the dietary choice of soil invertebrate herbivores has so far been examined only indirectly by, for example, observing changes in the cover of different potential food plants (Stein et al. 2010) or measuring the abundance of soil invertebrates next to host plant roots (Hemerik et al. 2003). A direct assessment of this interplay, however, is missing, raising the question whether feeding behavior of belowground herbivores is driven by their preference for particular plant species or by the availability of potentially palatable plant tissue, i.e., root abundance. The present study addresses this gap in our knowledge by experimentally testing the influence of plant identity and diversity on the dietary choice of soil insect herbivores.

We set up a mesocosm experiment focusing on wireworms, the larvae of click beetles (Coleoptera: Elateridae), as herbivores. Wireworms within the genus *Agriotes* are generalist herbivores, feeding on roots and tubers of different plant species (Traugott et al. 2008). *Agriotes* females preferentially lay their eggs in grassland soils, where larval development lasts 3–5 years (Parker and Howard 2001). While these wireworms can be highly abundant in grassland without causing damage, they are serious pests in a variety of crops such as maize, potatoes, or wheat (Parker and Howard 2001, Vernon 2005, Schallhart 2012). Their agricultural importance has led to an extensive body of literature on *Agriotes* biology and ecology. Furthermore, *Agriotes* larvae have been used in experiments examining the combined effects of below- and aboveground herbivory (Poveda et al. 2003, Wurst and van der Putten 2007). This makes them ideal herbivores for studying plant identity and diversity effects.

The feeding ecology of *Agriotes* larvae under natural conditions is best known in maize systems (Traugott et al. 2008). Maize fields typically harbor weeds, especially when organically cultivated. *Agriotes* larvae have been shown to consume both crops and weeds (Traugott et al. 2008). Here, we adopted the maize system to examine how plant identity and diversity affect elaterid food choice. Aside from maize, our experiment included three additional plant species typically found as weeds in arable land in the temperate zone, comprising the functional groups of grasses, legumes, and forbs. This combination simulates a situation *Agriotes* larvae often face in agricultural crop rotation. The larvae hatch in grassland, a plant-species-rich habitat with a well-established root felt. Prior to crop cultivation the area

is plowed, inducing a complete change in plant species composition for the developing larvae. The wireworms thus have to cope with an altered habitat containing fewer and different plant species.

An advantage of examining maize together with C₃ plant species is the applicability of stable isotope analysis. Maize, a C₄ plant, naturally differs in the carbon isotopic composition compared to the C₃ plants typical of temperate climates (Ehleringer and Rundel 1988). Based on these isotopic differences, the extent of plant consumption could be differentiated between the two plant groups. To distinguish wireworm feeding on different C₃ plants, nitrogen isotopic labeling was employed. Because the isotopic signal of consumed plants is incorporated in the consumers' tissues, the use of both natural and labeling-induced isotopic differences facilitates the direct and long-term tracking of herbivore feeding.

In our experimental setup, the four plant species (maize, perennial ryegrass, red clover, and gallant soldier) were offered to *Agriotes* larvae in different combinations and at varying diversity levels to test the following two hypotheses: (1) the dietary choice of *Agriotes* larvae is driven by a preference for specific plant species, i.e., the larvae will not feed on roots in proportion to their relative abundance, and (2) the preference for specific plant species changes with increasing plant diversity. We based the first hypothesis on the notion that most insect herbivores show selective feeding due to nutrient regulation and/or dilution of harmful secondary plant compounds (Waldbauer and Friedman 1991, Bernays et al. 1994), whereas the second hypothesis is rooted in the assumption that herbivores become more discriminating as their options increase (i.e., Scherber et al. 2006).

MATERIALS AND METHODS

The experiment was conducted in a climatic chamber (light 07:00–20:00; mean air temperature in light, 17°C, in darkness, 13°C). A special illumination system emitted photosynthetically relevant wavelengths and provided a photon flux density of approximately 600 μmol (lamps, Philips Master Colour CDM-T 830; Koninklijke Philips Electronics, Eindhoven, The Netherlands).

Four plant species were cultivated for the experiment: maize (*M. Zea mays*, Poaceae), perennial ryegrass (*G. Lolium perenne*, Poaceae), red clover (*L. Trifolium pratense*, Fabaceae), and gallant soldier (*F. Galinsoga ciliata*, Asteraceae). The latter species commonly grow in organic maize fields and represent three different functional groups: grasses (*L. perenne*), legumes (*T. pratense*), and forbs (*G. ciliata*).

We distinguished feeding on maize (a C₄ plant) from feeding on the other three C₃ plants via δ¹³C signatures because C₄ and C₃ plants naturally differ in their ¹³C content (Ehleringer and Rundel 1988). To distinguish between larval consumption of the grass, legume, and

TABLE 1. The plant and labeling combinations cultivated in the mesocosms.

Plant-labeling combination	Diversity level	Number of replications (mesocosms)	Number of <i>Agriotes</i> larvae analyzed
M G	I	5	24
M L	I	5	21
M F	I	5	19
M G _i L	II	5	22
M G L _i	II	5	23
M G _i F	II	5	20
M G F _i	II	5	21
M L _i F	II	5	20
M L F _i	II	5	17
M G _i L F	III	5	23
M G L _i F	III	5	20
M G L F _i	III	5	24
M	monoculture	5	24
G	monoculture	1	4
L	monoculture	1	4
F	monoculture	1	3
G _i	monoculture	2	7
L _i	monoculture	2	9
F _i	monoculture	2	8

Notes: Plant abbreviations are: M, maize; G, grass; L, legume; F, forb. Plants marked with a subscript i were isotopically labeled. M G_i L and M G L_i are the two labeling combinations of the same plant combination, for example. Monoculture mesocosms served as control groups. The experiment started with five *Agriotes* larvae per replicate, but some of them died or could not be found afterward. The number of analyzed *Agriotes* larvae therefore varies in different plant combinations.

forb, we enriched their ¹⁵N content by labeling individuals of all three species prior to the experiment. For isotopic labeling, plants were grown in an ¹⁵N-enriched ammonium nitrate solution (5.7 mg/L ammonium nitrate, 99 atom % ¹⁵N; Campro Scientific, Berlin, Germany) for 7 (grass and legume) and 8 (forb) weeks. The plant seeds were put in small pots (diameter: 5.8 cm, depth: 7 cm) filled with fine gravel. The substrate was moistened twice a week with the ammonium nitrate solution until saturation. Unlabeled plants were treated the same way using standard ammonium-nitrate solution (i.e., not enriched in ¹⁵N).

After labeling, the plants were carefully dug out, cleaned of gravel and transferred into mesocosms (rectangular pots, 15 × 15 × 20 cm length × width × depth) filled with sieved soil (Compo Sana Rasenerde, COMPO GmbH and Co. KG, Münster, Germany; sieve aperture 4 mm) and vermiculite (mixing ratio 5:1).

In the mesocosms, the four species were planted together in different combinations and at varying diversity levels (Table 1). At diversity level I (two plant species), maize was combined with an unlabeled grass, legume, or forb. At diversity level II (three plant species), maize was combined either with grass and legume, grass and forb, or legume and forb. At diversity level III, all four species were grown together. In mesocosms where two or more C₃ plants were present (diversity levels II and III), one of the C₃ plants was ¹⁵N-labeled for differentiation. This means that there were

different labeling combinations for one plant combination. For example, when maize, grass, and legume were put together, there were two different labeling combinations: one with ¹⁵N-labeled grass and one where the legume was labeled. Every labeling combination was replicated five times. Each plant species was also grown in a monoculture, where the labeled and unlabeled C₃ plants were maintained separately (Table 1).

The plant combinations were allowed to establish for 27 days before five *Agriotes* larvae (fifth to eighth instar) were added to each mesocosm. Larvae remained in the mesocosms for 25 days. The mesocosms were arranged randomly in the climatic chamber and repositioned twice during the experimental period to avoid biasing plant growth. The *Agriotes* larvae were exclusively collected from perennial grasslands in Tyrol, thus displaying isotopic signatures of a strict C₃ plant diet. They were kept in a climatic chamber in moistened soil. Three months prior to the experiment, the larvae were starved to ensure a high proportion of feeding-active individuals. All larvae were identified post mortem via a molecular assay (Staudacher et al. 2011). The vast majority was *Agriotes obscurus* (L.) (258 individuals), along with 38 and 17 larvae of *Agriotes sputator* (L.) and *Agriotes ustulatus* Schaller, respectively.

At the end of the experiment, all larvae were freeze killed, and their ninth abdominal segment was cut off and prepared for stable isotope analysis. The plants were carefully dug out and the roots cleaned of soil. They were photographed, dried for several days at 60°C, separated in above- and belowground tissues and their biomass determined to the nearest 0.01 mg. Root samples of all labeled and a subsample of unlabeled plants were prepared for stable isotope analysis.

Analysis

Carbon and nitrogen stable isotope ratios of all samples were determined at the Centre for Stable Isotope Research and Analysis (University of Göttingen, Göttingen, Germany). Atmospheric nitrogen and Pee Dee belemnite limestone were used as standards for nitrogen and carbon, respectively, and acetanilide (C₈H₉NO; Darmstadt, Merck, Germany) was used for internal calibration. Stable isotope signatures of samples were expressed in δ values (Peterson and Fry 1987). The original isotopic measurements of roots and wireworms are provided in Appendices A and B.

The root samples were analyzed to check for labeling success by looking for significant differences in the δ¹⁵N signatures between a mix of unlabeled roots from all plant species and pooled sets of labeled roots from grass, legume, and forb. Subsequently, the isotopic compositions of *Agriotes* larvae from monoculture mesocosms were checked for significant differences. A subsample of plants from every species was also analyzed for its root nitrogen content and checked for significant differences between plant species. Isotopic signatures of plants and wireworms, as well as the

nitrogen content of roots, were statistically analyzed using the Mann-Whitney *U* test (PASW Statistics 18; IBM, Armonk, New York, USA).

Mixing models (e.g., Phillips and Gregg 2001), which are normally used to calculate the proportion of different food sources in an animal's diet, were not applicable in the present case, because they assume that the isotopic signature of the consumer is derived by food that is available throughout the consumers' development. In the current experiment, however, the larvae were offered new food sources that were isotopically distinct to their previous C₃ plant diet and they performed a diet switch. As we know from previous feeding experiments (Traugott et al. 2007), the change toward the signature of the new food sources will not have been completed within the 25 days of the current experiment. Because of this, mixing models would underestimate the proportion of the new diet (maize or ¹⁵N-labeled C₃ plants) as the isotopic signatures of the wireworms still display high proportions of their former C₃ plant diet. Instead, we calculated thresholds to identify the larval diet of diversity level I–III, by adding 1.96 × the standard deviations to the arithmetic means of the isotopic signatures of the larvae in the monoculture mesocosms. We only used larvae of monocultures containing C₃ plants (labeled and unlabeled) to calculate the δ¹³C threshold, and only larvae of monocultures containing unlabeled plants (mesocosms of all four plant species) to calculate the δ¹⁵N threshold. Animals of diversity level I–III, displaying isotopic signatures below the thresholds, were designated to have fed exclusively on C₃ plants (δ¹³C threshold) or unlabeled plants (δ¹⁵N threshold). Likewise, individuals with signatures above the thresholds were assigned to have fed on maize (δ¹³C) or labeled plants (δ¹⁵N).

The thresholds were used to implement a categorical variable displaying whether the isotopic signature of a larva was below (value of variable = 0) or above (value = 1) the corresponding threshold. This variable was used to calculate the percentages of larvae feeding on specific plant species in all plant combinations.

To assess wireworm dietary preferences, we first calculated the mean proportion of available root biomass for the different plant species in all plant combinations. Plants with severely damaged roots were omitted in these calculations since information on plant root availability prior to consumption is required for calculating Manly's alpha preference index. An in situ assessment of the root biomass prior to the feeding experiment was impossible without severely disturbing the mesocosms.

We used Manly's alpha preference index for constant prey populations (described in Krebs 1989) to calculate the preference of wireworms toward the different plant species

$$\alpha_i = (r_i/n_i) \times \left[1 / \sum (r_j/n_j) \right]$$

where α_i = Manly's alpha preference index for prey type *i*; r_i and r_j are the proportion of prey type *i* or *j* in the diet (in our case, the proportion of wireworms feeding on roots of plant types *i* or *j*; i and $j = 1, 2, 3, \dots, m$); n_i and n_j are the proportion of prey types *i* or *j* in the environment (in our case, the proportion of the root biomass of plant types *i* or *j*); and m = number of prey (in this case, plant) types possible.

This index provides a value of relative preference by comparing it to $1/m$ (m = total number of plant species in the mesocosm). If α_i is greater than $1/m$, then the plant species is preferred; if α_i is less than $1/m$, then the plant species is avoided. Therefore, the $1/m$ notation is referred to here as the preference–avoidance threshold. Note, however, that the values for α_i are normalized ($\sum \alpha_i = 1$), meaning that the sum of all α_i values is constant. Therefore, if one plant species is preferred, another one has to be avoided. For calculating Manly's alpha indices, wireworm and root data were pooled among replicates.

Since grass, legume, and forb were not labeled in diversity level I, the proportion of these plants in the larval diet (r_i, r_j) could not be assessed, thus making it necessary to adopt the calculation of Manly's alpha indices for maize in diversity level I as follows:

$$\alpha_m = r_m/n_m \times 0.5$$

where α_m = preference index for maize; r_m = actual proportion of larvae feeding on maize; and n_m = actual proportion of root biomass of maize.

Since there were two food sources in diversity level I, the $1/m$ value is 0.5. A preference index below 0.5 indicates the avoidance of maize, whereas an index above 0.5 indicates preferential feeding on maize. Note that this index is not normalized and therefore can reach values above 1.

RESULTS

Root and larval signatures in monocultures

The δ¹⁵N signatures of roots of all labeled individuals of grass (286.7 ± 704.5 [mean ± SD], $n = 30$ individuals), of legume (503.9 ± 573.4, $n = 29$ individuals), and of forb (36.3 ± 48.2, $n = 30$ individuals) were significantly higher than the root signatures of the unlabeled plants (−1.5 ± 2.3, $n = 40$ individuals) or of the soil (3.0 ± 4.1, $n = 14$ samples; $P < 0.001$ for all three tests).

Likewise, ¹⁵N signatures were significantly higher in larvae from monoculture mesocosms with labeled plants (27.7 ± 19.8, $n = 24$ larvae) compared to those of larvae fed with unlabeled plants (3.6 ± 1.8, $n = 35$ larvae). The ¹³C signatures of larvae fed with maize (−22.6 ± 2.5, $n = 24$ larvae) differed significantly from larvae kept in pots with C₃ plants only (−26.4 ± 1.6, $n = 35$ larvae; $P < 0.001$ for both tests). The thresholds used to assign larvae to feeding on labeled plants and maize at diversity levels I–III, were 7.063‰ for δ¹⁵N and −23.498‰ for δ¹³C ($n = 35$ larvae for both thresholds).

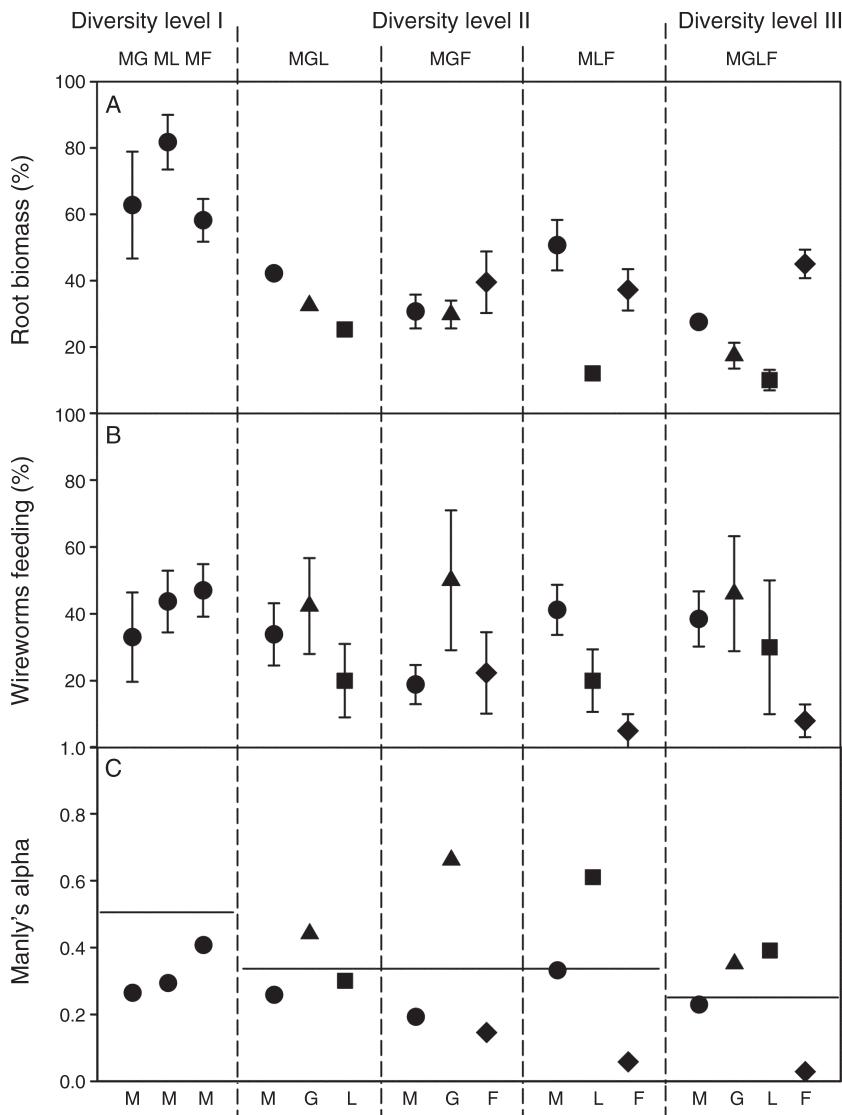


FIG. 1. (A) Mean percentage of root biomass of different plant species, (B) mean percentage of wireworm *Agriotes* larvae feeding on different plant species, and (C) Manly's alpha indices at different plant combinations and diversity levels. Plant combinations are displayed on top, and species are displayed at the bottom of the figure: maize, M (circle); grass, G (triangle); legume, L (square); forb, F (diamond). Note that only data for M are available at diversity level I. Solid horizontal lines in panel C show preference-avoidance thresholds for Manly's alpha indices.

Nitrogen content and biomass of plant roots

The roots of the legume had the highest relative nitrogen content ($2.9\% \pm 0.3\%$), which was significantly higher compared to grass roots ($2.1\% \pm 0.2\%$; $P = 0.002$), whereas the nitrogen contents in roots of the forb ($2.5\% \pm 0.6\%$) and maize ($2.3\% \pm 0.5\%$) were intermediate.

At diversity level I, maize made up for most of the plants' root biomass, ranging between 58% and 82%, whereas, at diversity levels II and III, the relative proportion of maize roots decreased to 31–51% and 28%, respectively (Fig. 1A). The forb accounted for 37–42% of the total root biomass across the first two

diversity levels and produced the largest root system at diversity level III (45% of total root biomass). The relative root biomass of the grass ranged between 30% and 37% in the first two diversity levels and decreased to 17% in level III. The root system of the legume was the smallest of all four plant species ranging between 10–25% of the total root biomass (Fig. 1A).

Larval dietary choice

Eighty-five percent of all larvae used in the mesocosms were recovered after the experiment, evincing low mortality. The percentage of *Agriotes* larvae feeding on maize over all diversity levels and plant combinations was 40%. Values ranged between 33% and 48% at

diversity level I, between 20% and 46% at diversity level II, and 40% for diversity level III (Fig. 1B).

The overall percentage of larvae feeding on the forb was 11%, with 6% and 19% at the two combinations of diversity level II, and 8% at diversity level III (Fig. 1B).

The percentage of larvae feeding on the grass was 48% over all combinations, 41% and 65% at diversity level II, and 39% at level III (Fig. 1B). The percentages of larvae feeding on the legume were 20% and 22% at diversity level II and 25% at diversity level III (overall 22%; Fig. 1B).

Larval dietary preferences

Grass and legume were the most preferred plants at all diversity levels, with preference indices averaging 0.18 (grass) and 0.13 (legume) higher than the preference–avoidance threshold (Fig. 1C). These similar preference indices were derived differently: in the grass the high preference index resulted from large numbers of *Agriotes* larvae feeding on this moderate root-mass plant, whereas the high values for the legume were derived from a moderate number of larvae feeding on this very low root-mass plant. In all types of combinations and at all diversity levels where grass and legume were planted together, a decrease of their preference index could be observed compared to plant combinations harboring only one of these species. Both maize and forb were avoided, with indices averaging 0.11 and 0.23 below the preference–avoidance threshold, respectively (Fig. 1C). By far the lowest preference indices were found for the forb (Fig. 1C), with comparatively few larvae feeding on this plant despite its high root mass. In all plant combinations where the forb was present, the preference indices of the other plants were higher compared to non-forb treatments, except for maize consumption in mesocosms MGL and MGF (Fig. 1C).

Generally, *Agriotes* preference for and avoidance of all four plant species was constant over all diversity levels. However, we found distinct changes in the extent of preference and avoidance with increasing plant diversity: the preference for maize and legume increased strongly from diversity level II, when planted in combination with grass (MGL), to diversity level III, where the forb was added. When maize and legume were planted together with the forb at diversity level II (MLF), their preference index decreased at diversity level III, when the grass was added (Fig. 1C). Moreover, for maize the extent of avoidance decreased with increasing plant diversity: maize was least preferred at diversity level I (0.18 below the threshold), whereas preference was close to the preference–avoidance threshold at diversity level III (i.e., 0.02 below the threshold).

DISCUSSION

Our hypotheses were (1) that the dietary choice of *Agriotes* larvae is driven by preference for certain plant species rather than by root abundance, and that (2) the preference for specific plants changes with increasing

plant diversity. Our results support the first hypothesis, revealing that plant identity (i.e., plant-specific traits) drive the feeding choices of these soil insects. Furthermore, our results also suggest that plant diversity can modulate the degree of preference for specific plant species.

Generalist herbivores such as *Agriotes* larvae have two potential advantages compared to specialists: greater resource availability and the capability of mixing foods to improve nutrient balance and reduce intake of toxic allelochemicals. For aboveground generalist herbivores, Bernays and Minkenberg (1997) revealed differences in the effect of mixed diets on survivorship, growth, and fecundity between two groups of insects: Lepidopteran larvae and Orthoptera. While a mixed diet had a positive effect compared to single-food diets in the Orthoptera, no such effect was present in Lepidoptera. The authors assumed that the high mobility of the Orthoptera allows them to benefit from plant diversity by nutrient mixing, whereas for the less mobile lepidopteran larvae the advantage of being a generalist herbivore primarily lies in greater resource availability, reducing the risk of starvation due to an insufficient supply of food plants. Accordingly, in belowground systems, where locating and acquiring specific plant species is hampered by the herbivores' restricted movement (Maraun et al. 2003), generalist feeding behavior appears to be of greater advantage than mixing foods. This is especially important for larvae with a longer development in the soil. The *Agriotes* larvae investigated here consumed all plants offered in the experiment, indicating a utilization of a broad food spectrum, within which selective feeding occurs, as indicated by the preference indices.

Organisms require an array of different nutrients to complete their life cycle (Behmer 2009). Since nutrient composition varies considerably among plant species, most generalist herbivores feed selectively on different plants to receive the appropriate mixture (Schoonhoven et al. 2005, Behmer 2009). In *Agriotes lineatus* larvae, for example, survival rates ranged between 3% and 50% when fed on different host plants (Kabanov 1975). This has been attributed to different nutrient composition, although the potential effect of secondary plant compounds was not taken into account. Nitrogen is a crucial nutrient for herbivores (Mattson 1980). Legumes provide high amounts of nitrogen for root feeders, and C₃ grasses usually have higher nitrogen contents than C₄ grasses (Mattson 1980). The dietary preference observed in this study, however, can only be partly explained by plant nitrogen content. While the roots of the legume had the highest nitrogen content, grass, the other preferred plant in our experiment, had the lowest mean values.

Beside nutrient content, chemical deterrents also affect herbivore diet choice. Our finding that wireworms strongly avoided *G. ciliata*, for instance, may result from sesquiterpene lactones produced by most species belonging to the Asteraceae. Landau et al. (1994) found that cnicin, a

sesquiterpene lacton of the Asteraceae *Centaurea maculosa*, was strongly toxic to the larvae of a generalist noctuid moth. Moreover, Dawson et al. (2002) found that grass grubs (*Costelytra zealandica*) grew better when feeding on *Trifolium* than on other legumes (Prestidge et al. 1985, Gaynor et al. 1986). The authors attributed this to the low concentrations of chemical deterrents in *Trifolium*. The preference for the legume observed in our experiment might thus be ascribed to a combination of high nitrogen availability and low deterrent content.

The legume and the grass were the preferred food plants of the wireworms in the current mesocosm experiment. In accordance with the results of our study, Fox (1973) identified grasses and clover as the preferred food sources of *A. obscurus* larvae compared to moss, yarrow, and different dandelion species in perennial grassland. Likewise, Wardle et al. (2003) measured the highest densities of herbivorous nematodes in grasses and the lowest in forbs, with intermediate densities in legumes. The outcomes of these studies are based on spatial proximity to the host plants. Therefore, both plant quality and the species-specific microhabitats they produce can play an important role for the preference or avoidance of a specific plant as food source. We assume that the influence of the microhabitat can be neglected in our experiment. First, wireworms are larger than nematodes, making them less affected by small-scale differences in the root habitat. Second, the roots of the different plant species were interwoven in the mesocosms, meaning that large areas of species-specific root habitats were unlikely to be present in the current experiment.

The nutrient- and deterrent-based theory of plant preference and avoidance in generalist herbivores presupposes that *Agriotes* larvae are type A generalists, i.e., all individuals feed on a variety of different plant species instead of each individual specializing on a different species (Van Valen 1965, Grant et al. 1976). However, ¹³C signatures of *A. obscurus* larvae collected in a weedy maize field indicated that in this wireworm species both types of generalists are present in a population, but type A generalists dominated (Traugott et al. 2008).

In regard to our hypothesis that plant diversity affects the dietary choice of *Agriotes* larvae, we found that the general preference (grass and legume) and avoidance (forb and maize) for different food types were constant across diversity levels. Interestingly, the extent of preference and avoidance changed with increasing plant diversity. The direction of these changes was defined by the preference for the added plants. Adding highly preferred (grass in our case) or highly avoided (forb in our case) plants decreased or increased the other plants' preference indices, respectively. With increasing diversity, preference patterns become more complex: in our experiment, the level of avoidance toward maize decreased with increasing plant diversity. This could be explained by the forb preferentially affecting the maize preference index (i.e., increasing it), whereas the other two plants affected each other by being similarly preferred by the wireworms (i.e., leading to a

reduced preference index of both plants). Note, however, that the mesocosms established here simulated a maize field situation with a restricted number of typical plant species. To investigate the influence of plant diversity in detail, a more comprehensive analysis, comprising more diverse plant communities, will be required.

Our experiment also has implications for pest management. Soil herbivores can wreak havoc on many crops, especially in monocultural systems where herbivores cannot choose between different plants. Trap crop experiments, where pests such as wireworms are lured away from the main crop, revealed a significant reduction in crop damage (Vernon et al. 2000, Shelton and Badenes-Perez 2006). Our results suggest that the selection of the trap crop species will critically affect the success of this approach due to the divergent preference for different plant species.

In conclusion, we demonstrated that plant species identity affects the dietary preferences of *Agriotes* larvae and that plant diversity can influence the extent of preference. Root abundance played only a minor role, but may be important if wireworms chose between food plants with similar nutrient composition and digestibility. The nitrogen content of roots only partly explained wireworm feeding behavior, suggesting that food choice in soil insect herbivores is driven by a combination of different traits.

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SUPPLEMENTAL MATERIAL

Appendix A

Tables and figures displaying the original isotopic signature of every single *Agriotes* larva analyzed in the experiment. Separate figures are provided for every plant–labeling combination (*Ecological Archives* E093-246-A1).

Appendix B

Tables displaying the original isotopic data of unlabeled plants and labeled plants in the experiment (*Ecological Archives* E093-246-A2).