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Short Communication

Stable isotope analysis reveals whether soil-living elaterid larvae move between agricultural crops

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Tracking the movement of soil-living herbivores is difficult, albeit important for understanding their spatial ecology as well as for pest management. In this study the movement of *Agriotes obscurus* larvae between plots harbouring isotopically different plants was examined. Neither between maize and wheat nor between maize and grassland movement could be detected. These data suggest that *Agriotes* larvae rarely disperse between crops as long as local food supply is sufficient. Moreover, the current approach provides a new means to study the dispersal of soil invertebrates *in situ*.

The larvae of click beetles (Coleoptera: Elateridae) are commonly called wireworms. Within the genus *Agriotes* they are soil-living and feed on a variety of plants including arable crops (Parker and Howard, 2001; Traugott et al., 2008). The opaque habitat these wireworms live in hampers direct observation, including the assessment of their dispersal behaviour between crops. The latter, however, has important implications for managing these pests. In the present study we present a new approach to study the dispersal behaviour of these insects using the analysis of stable isotopes.

The study site was located in Weer (Tyrol, Austria) comprising two plots of maize (*Zea maize*); one bordered by a plot of wheat (*Triticum aestivum*) the other by permanent grassland. Maize and wheat plots were 3.5 × 20 m and newly established within the permanent grassland on 15 June 2009. The maize plot next to the wheat was manually weeded, whereas the other maize plot included some C3-weeds. As wireworms incorporate the isotopic signatures of the plants they feed on, movement between plots of plants with different isotopic signatures can be traced (Schallhart et al., 2009). To investigate the movement from the maize into the wheat plot and the grassland, respectively, the natural differences in $\delta^{13}C$ signatures between C3-plants (wheat and grassland plants) and C4-plants (maize) were used. To identify larvae moving from the grassland into the maize plot, the grassland vegetation was treated with a ^{15}N-enriched ammonium nitrate-solution (85.7 mg/m^2 ammonium nitrate, 99 atom% ^{15}N; Campro Scientific, Germany) on 17 June 2009. Between 12 and 30 August 2009, 143 *Agriotes* larvae were collected within the following transects: along the borderline between the crops and at distances of 30 and 60 cm parallel to the borderlines in both directions. Thirty-seven individuals of the collected *Agriotes* larvae were instar 1–4, 106 were instar 5–8. Fifty percent of the specimens collected were identified to species level via molecular means (Staudacher et al., 2011); all of them were *Agriotes obscurus* (L.). The animals were freeze-killed upon collection and the 9th abdominal segment was subjected to isotope analysis.

Additionally, root samples were taken from the grassland 28 days after labelling (two samples each from the borderline as well as 30, 60 and 90 cm deep into the grassland). All samples were dried and weighted for isotopic analysis and measured for their ^{13}C- and ^{15}N-content at the Kompetenzzentrum Stabile Isotope, University of Göttingen (Germany). Isotopic thresholds were established based on data derived from a feeding experiment where *Agriotes* larvae fed exclusively on one of three plant diets (maize, ^{15}N-labelled C3-plants or unlabelled C3-plants) for 25 days. The thresholds were calculated by adding two times the standard deviation to the arithmetic mean of the larvae's signatures (i.e. covering ~97.5% of the values), establishing a threshold for $\delta^{13}C$

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of −23.3‰ ($n = 35$; larvae fed on labelled and unlabelled C3-plants) and for $\delta^{15}N$ of 7.1‰ ($n = 35$, larvae fed on maize and unlabelled C3-plants). Field-caught larvae with signatures exceeding these thresholds indicated either a maize-diet ($\delta^{13}C$) or feeding on labelled grassland plants ($\delta^{15}N$).

The $\delta^{15}N$ signatures of root samples from the labelled grassland were significantly enriched (13.7‰ ± 5.0; mean ± SD) compared to the pooled root samples from the border and the unlabelled plant roots of the feeding experiment (−0.7‰ ± 3.2; $p = 0.002$, $U < 0.001$; Mann–Whitney U test). Accordingly, the $\delta^{15}N$ signatures of *Agriotes* larvae from the labelled grassland were significantly higher (13.8‰ ± 6.4) compared to those from the border and the maize plot (4.1‰ ± 1.6; $p < 0.001$, $U = 21.0$; Mann–Whitney U test), allowing to distinguish between larvae feeding on maize and on plants within the labelled grassland. Several specimens with $\delta^{15}N$ signatures above the $\delta^{15}N$ threshold were found in the grassland (100% and 79% at 60 cm and 30 cm transects, respectively) and at the crops' borderline (10%), whereas this was true for only one larva, found 60 cm deep in the maize (Fig. 1). No larvae were found in the grassland which surpassed the $\delta^{13}C$ threshold, indicating that no, or only few, wireworms migrated from the maize into the grassland (Fig. 1). Between the maize and wheat plots, *Agriotes* larvae with $\delta^{13}C$ signatures indicating a maize-diet were found only at the crop border as well as 30 cm and 60 cm deep in the maize (21%, 59% and 90%, respectively), whereas no such individuals were found in the wheat plot (Fig. 2).

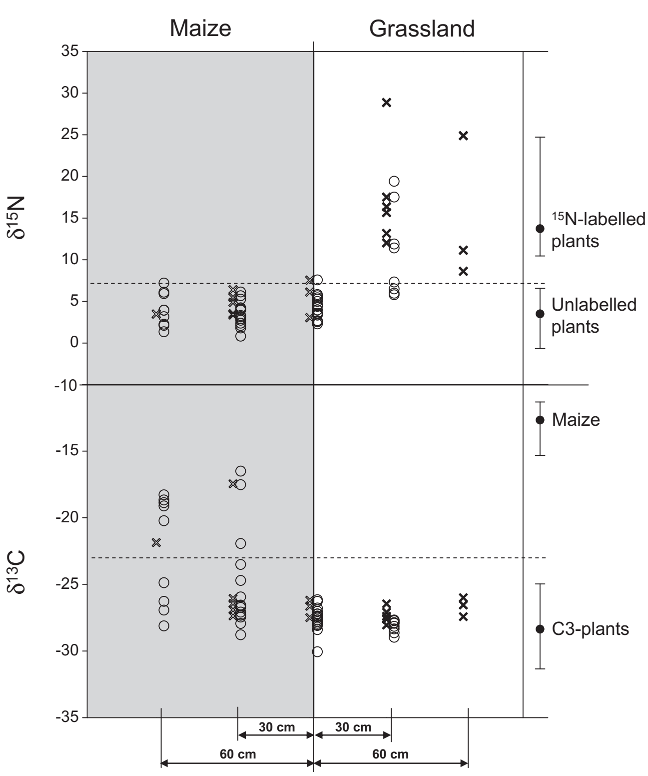

Fig. 1. $\delta^{15}N$ and $\delta^{13}C$ signatures of *Agriotes obscurus* larvae collected at different distances between a maize plot and grassland. Each symbol (X L1–L4, circle L5–L8) represents the isotopic signature of a single specimen. Signatures of early and late instar larvae are staggered for clarity. Note that samples with similar signatures can overlie each other. The dashed lines indicate the thresholds to differentiate between larvae feeding on C3-plants and maize and between unlabelled and labelled plants, respectively. Additionally, means of root samples are provided on the right hand side; error bars indicate minimum and maximum signatures; ^{15}N-labelled plants ($n = 6$), unlabelled plants ($n = 190$), maize ($n = 36$) and C3-plants ($n = 154$).

Fig. 2. $\delta^{13}C$ signatures of *Agriotes obscurus* larvae collected at different distances between a maize and wheat plot. Each symbol (XL1–L4, circle L5–L8) represents the isotopic signature of a single specimen. Signatures of early and late instar larvae are staggered for clarity. Note that samples with similar $\delta^{13}C$ signatures can overlie each other. The dashed line indicates the threshold to differentiate between larvae feeding on wheat (below) and maize (above). Additionally, means for wheat and maize samples are provided on the right hand side; error bars indicate minimum and maximum signatures of maize ($n = 36$) and wheat ($n = 18$).

For both crop combinations we found no movement between plots (ignoring the one specimen which just passed the threshold), although wireworms can cover distances up to 240 cm, depending on species, soil- and cultivation-type (Dobrovolsky, 1970; Arakaki et al., 2010). It has been proposed that wireworms show directional movement at small scales (10–20 cm, Dobrovolsky, 1970) and that *Agriotes* larvae are effectively lured away by trap crops (Vernon et al., 2000). Hence, we ascribe the lacking movement of the *A. obscurus* larvae in the current experiment to a sufficient on-site food supply, making movement between crops unnecessary. The current data also suggest that it is unlikely that *Agriotes* larvae invade into vulnerable crops, given that they are not food-limited. The dispersal of adult click beetles, where ∼20% of beetles collected in grasslands originated in an adjacent maize field, however, is much greater (Schallhart et al., 2009). Therefore, the dispersal of agrioted beetles from infested into unaffected fields can rather be ascribed to the adults than their soil-living larvae.

Within this study a labelling-by-feeding approach has been utilized to identify wireworms moving between isotopically distinct plant communities. The duration the food is offered and the quantity of food consumed by wireworms affect this approach. For example, *Agriotes* larvae switching from a C3- to a C4-plant diet will display the isotopic signature of the new diet as early as 3 weeks after the diet switch (Traugott et al., 2007). Hence, wireworms were collected several weeks after experimental set-up (maize: 59–108 d; grassland: 57–106 d), allowing them to incorporate the new isotope signatures. Feeding-inactive larvae, however, will not take up the isotopic signature from the plant habitat they live in. In both maize plots, 49% of larvae did not show a maize $\delta^{13}C$ signature, which indicates little or no feeding on maize plants. These larvae might have been starving or fed on the C3 grassland vegetation, ploughed in prior to the experiment. Moreover, wireworms which have moved between plots short after the isotopically distinct plant communities have been established will not be identifiable as crop-movers. Nevertheless, the current approach allows studying the dispersal behaviour of soil insects in their natural habitat, circumventing potential bias such as agitation dispersal induced by conventional mark-recapture experiments (Turchin, 1998). As Prasifka et al. (2004) have shown for carnivorous insect in above-ground systems, this approach might also be used to examine the movement of higher trophic level soil invertebrates.

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