



## Distinct macroinvertebrate soil food webs at one-meter scale in a Mediterranean agroecosystem

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### ABSTRACT

Soil macroinvertebrates play a central role in ecosystem processes such as decomposition and nutrient cycling, but the extent to which macroinvertebrate food web structure varies within ecosystems is basically unknown. Here, we examined changes in plant and macroinvertebrate community composition at increasing distances from trees within a citrus grove, following a gradient in organic matter and water availability created by the localized fertilization and irrigation of citrus trees. In addition, we analysed stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of soil, plants, and macroinvertebrates to investigate changes in trophic structure within the grove. The macroinvertebrate community near the tree trunks had a higher number of detritivores and predators than the community located farther away. After correcting the isotope values of macroinvertebrates by their isotopic baseline, a spatial shift in trophic structure emerged: macroinvertebrates near the trunks had lower  $\delta^{13}\text{C}$ -values than those located farther away (i.e., they apparently relied more on plant-based resources). This pattern is attributable to species only found near the trunks. In contrast, species distributed across the studied area showed no differences in their isotopic signatures across space, suggesting that they are connecting otherwise two compartmentalized food webs. A reduction in organic matter and water availability at increasing distances from trees is the most parsimonious explanation of the observed isotopic gradient in soil and plants, and this may, in turn, be responsible for the observed changes in food web composition and structure. This study shows that spatial variability in macroinvertebrate soil food webs can be present at scales as small as one meter if spatial variability in environmental factors is high enough.

### 1. Introduction

Macroinvertebrates play a crucial role in decomposer food webs. Many species act as ecosystem engineers: structuring the soil, processing large amounts of litter, and producing faeces that stimulate microbial activity and, therefore, decomposition (Hättenschwiler et al., 2005; Cole et al., 2006). Thus, by analysing the spatial variation in macroinvertebrate food webs and linking observed changes with environmental influences, it is possible to identify the key factors of the food web structure and ecosystem functioning (de Vries et al., 2013). As an example, due to habitat changes, macroinvertebrate communities and trophic structure can change, particularly in relation to the trophic guilds or energy pathways present (Seeber et al., 2005; Klarner et al., 2014).

The studies cited above focused on soil macroinvertebrate food web changes among ecosystems (see Hyodo (2015) for a review). This

approach considers all the species in an ecosystem as potentially interconnected through trophic interactions (community webs *sensu* Cohen (1977)), but the scale of the study and the scale at which organisms experience the environment is not necessarily the same. Even within the same ecosystem, some spatial variability in resources is expected, which can produce changes in food web composition and structure at small scales. Spatial patterns in relatively uniform ecosystems have been found in the microbiota (Ettema and Wardle, 2002), but when larger components of detrital food webs are included, horizontal patterns may disappear (Berg and Bengtsson, 2007). Conversely, in arid ecosystems, where spatial heterogeneity is high, certain microhabitats such as shrubs and nest mounds harbour more complex macroinvertebrate communities than their surroundings do (Doblas-Miranda et al., 2009). Similarly, Goncharov et al. (2014) found that different microsites around fallen trees harbour macroinvertebrate communities with different trophic structures.

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Soil food webs are often divided into grazing and detritus-based energy channels (Moore and de Ruiter, 1991). Rather than being strictly separated in discreet trophic layers, the position of feeding groups of detritivores and predators form a trophic continuum within a food web. Many species engage in opportunistic feeding behaviour, with the result that omnivory is a pervasive feature in these communities (Scheu, 2002). The formalization of such complex networks has traditionally been based on direct observations, but this kind of data is difficult to gather in soil communities because of the small size and cryptic habits of the species involved. Besides, occasional observations might not be truly representative of the long-term relationships between species.

The analysis of stable isotope ratios of C and N ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) is an alternative approach that integrates information over time regarding sources of energy used by animals, trophic positions, and trophic links among community members (Tiunov, 2007). Today this technique is fundamental in the study of soil food webs (Potapov et al., 2019; Quinby et al., 2020). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  increase with trophic level as a result of fractionation, a process by which consumers become enriched, compared to their diet, in the heavy isotope (trophic enrichment). While enrichment in  $\delta^{13}\text{C}$  is small ( $< 1\%$ ) and, therefore, useful for inferring potential food sources, the increase in  $\delta^{15}\text{N}$  is larger (approximately 3.4‰) and can be used to estimate trophic position (McCutchan et al., 2003; Potapov et al., 2019). Overall, the isotopic variability of a population can be used as a proxy for the width of the species trophic niche (Bearhop et al., 2004; Newsome et al., 2007), and has proved useful for elucidating trophic relationships between different components of the soil food web (e.g., Scheu and Falca, 2000; Ponsard and Arditì, 2000).

Here we assess the spatial variability in the soil macroinvertebrate food web of a Mediterranean agroecosystem. In particular, we wanted to understand the influence of spatial variability in environmental factors on the composition of the macroinvertebrate community and its trophic structure. To do so, we took advantage of a pronounced gradient in organic matter and water availability in a citrus grove caused by localized fertilization and irrigation beneath the tree canopies. Across this gradient we i) analysed the plant and macroinvertebrate community composition, ii) characterized the C and N stable isotopes of soil, plants, and macroinvertebrates, and iii) defined trophic groups of species within the delimited food webs. Food web delimitation was based on differences across space in macroinvertebrate community composition and stable isotope signatures. We hypothesized that changes in food web composition and structure would emerge at the scale at which changes in resource availability were most important. Specifically, we expect the sites beneath the tree canopies to constitute a local refuge for soil fauna in an otherwise semiarid system, driving changes in the identity, abundance, and trophic relationships between soil organisms in comparison to sites located further away.

## 2. Materials and methods

### 2.1. Study site

The study was conducted in an organic citrus grove located in La Selva del Camp (Tarragona, NE Spain; 41°13'07"N, 01°08'35"E). The grove consists of ca. 300 clementine trees (*Citrus clementina* var. *clementines*) grafted on the hybrid rootstock Carrizo citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.). Trees are watered twice a week during dry periods and fertilized each winter with organic manure. Grasses and other weeds form a permanent ground cover, which is mowed three or four times a year. More information about the study site and the arthropod assemblages present in the grove can be found in Piñol et al. (2012).

### 2.2. Experimental design and sampling details

We selected for sampling 9 adjacent trees within the citrus grove,

arranged in 3 rows and 3 columns. Trees within a row are separated by 3.5 m and rows are 6 m apart. Since irrigation and fertilizer are applied beneath the tree canopies, we sampled at three distances from each tree: Trunk (20 cm to the tree trunk, just beneath the canopy), Middle (150 cm distant, at the outer limit of the canopy), and Row (350 cm distant, between rows); thereafter referred as treatments. Thus, we generated 27 samples in total. Fig. A.1 shows a detailed layout of the experimental design.

Samples were taken in July 2013 between 6 a.m. and 10 a.m. At each sampling location, we collected the aerial parts of all plants over an area of 25 × 25 cm<sup>2</sup> and preserved them in a dry paper for further identification and processing. There was not a clear developed layer of plant litter, but fresh and dried plant material was standing on the soil. For this reason, we directly dug the area to a depth of 15 cm and extracted the bulk of soil.

The soil was torn apart in small quantities over a white plastic tray, all macroinvertebrates were collected with pincers and entomological aspirators and immediately preserved in 70 % ethanol. Soil macrofauna can be sampled by hand sorting, and taxonomic groups are typically around 2 mm body width (Swift et al., 1979; Coleman et al., 2004). For this reason, mesofauna was not considered here. Despite most species of mites are associated to mesofauna, three morphospecies of large predatory mites (1–3 mm body width) were considered in this study. We kept 1 kg of each soil sample for future analysis.

We also collected leaves from each sampled citrus tree and samples of the fertilizer applied over the last three years (2011, 2012, 2013). The fertilizer of 2013 had a different isotopic composition from that of the other years because of an obligate change of provider.

### 2.3. Sample processing and stable isotope analysis

Soil samples were milled and sieved to 0.2 mm and pulverized on a mortar grinder. We analysed organic matter by oxidation and eliminated inorganic carbon from soil by acidification following Hesse (1971) and Midwood and Boutton (1998), respectively.

Plants were identified using a reference collection of the studied area (Kindermann, 2010) and with keys from Bolòs et al. (2005). We selected for isotopic analyses those species accounting for 90 % of the total biomass of each sample and homogenized them to a fine powder with a mixer mill.

Macroinvertebrates were identified to the species level when possible. We selected for isotopic analyses members of edaphic species for which individuals of the same life stage were present at least in two samples of a treatment (for ants we considered that a nest was present if there were at least 25 homospecific workers). Analysed species amounted to 48 % of the total species richness, which encompasses 81 % of the total individuals abundance.

We weighed 8–10, 1–1.5, and 0.2–2.5 mg of processed soil, plant, and macroinvertebrates, respectively, into tin capsules for stable isotope analyses. If an invertebrate specimen weighed more than 2.5 mg, we homogenized it to a fine powder with a pestle. For snails and earthworms, we only analysed muscle tissue. Samples were dried for 24–48 hours at 60 °C.

We analysed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in all samples at the Stable Isotopes Analysis Lab (LAIE) at the Autonomous University of Barcelona, using a continuous-flow Delta V Advantage Isotope Ratio Mass Spectrometer, coupled to a Flash 1112 elemental analyser (both from Thermo Fisher Scientific Inc., Waltham, MA USA). Samples were standardized to IAEA-600 (caffeine,  $\delta^{13}\text{C}$ :  $-27.771\text{‰} \pm 0.043$  SD;  $\delta^{15}\text{N}$ :  $1.0\text{‰} \pm 0.2$  SD) from the International Atomic Energy Agency (Vienna, Austria). Carbon and nitrogen isotope ratios were calculated relative to VPDB and atmospheric air, respectively, and expressed as ‰.

In total, we performed 377 stable isotope analyses (27 of soil and 9 of fertilizer, 79 of plants, including citrus leaves, and 262 of macroinvertebrates), comprising 18 species of plants and 39 species of macroinvertebrates.

## 2.4. Data analyses

All analyses followed a randomized block design, with treatment (each distance class to the tree trunk) as a fixed factor and tree as a random factor (each tree containing three samples, one for each treatment).

### 2.4.1. Plant and macroinvertebrate communities

To assess spatial variability in food web composition, we compared the plant and macroinvertebrate communities between treatments, analysing species composition and abundance of taxonomic groups (family or higher). We used biomass ( $\text{g m}^{-2}$ ) and count data (individuals  $\text{m}^{-2}$ ) for plants and macroinvertebrates, respectively. Despite soil samples consisted in volume rather than surface, we keep our measures expressed as individuals  $\text{m}^{-2}$  because 1) soil fauna abundance is often expressed in these units and we aim to keep our variable comparable to other similar studies, 2) we analysed the bulk of soil as a whole in relation to macrofauna, and 3) a systematic transformation would not modify the results (*i.e.* the depth was the same for all samples). For community composition, raw data were square-root transformed, and a permutational multivariate ANOVA was performed using the Bray-Curtis dissimilarity index. If differences among treatments were significant, we ran a *posteriori* pairwise comparisons. For the analyses of the abundance of taxonomic groups, we conducted a permutational ANOVA using Euclidean distances between samples, and Monte Carlo *P*-values were generated when the number of possible permutations was less than 100. *P*-values were adjusted following the False Discovery Rate (FDR) method. If differences among treatments were significant, we ran a *posteriori* pairwise comparisons.

We analysed the relationship between plant and macroinvertebrate communities with a Mantel test, using the Bray-Curtis dissimilarity index between samples and the Pearson correlation.

To visualize differences in macroinvertebrate community composition among treatments we conducted a non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity index. To detect which macroinvertebrate species differed between treatments, we calculated the contribution of each macroinvertebrate species to the observed dissimilarity with a SIMPER analysis.

### 2.4.2. Stable isotopes of soil, plants and macroinvertebrates

To assess spatial variability in food web trophic structure, we compared the stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of soil, plants and macroinvertebrates among treatments. For soil and plants, we conducted a permutational ANOVA using Euclidean distances between samples. For plants, we included species as a random factor nested in family (random factor).

For macroinvertebrates we ran a permutational ANCOVA on macroinvertebrate stable isotope signatures with soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as covariates, that is, macroinvertebrates of a given sample had the soil signature of the same sample as a covariate. This corrected community signature by trophic baseline and then determined if trophic shifts were present among treatments. This approach is normally used in soil food web studies (Potapov et al., 2019), and in our study is supported by the fact that almost all macroinvertebrate signatures are close to those of the soil. We set the trophic guild of macroinvertebrates as a random factor. The trophic guild of macroinvertebrates (detritivores, predators, herbivores and omnivores) was defined following the existing literature (Armengol, 1986; Blas, 1987; Altaba and Ros, 1991; Lövei and Sunderland, 1996; Deckle and Fasulo, 2001; Zimmer, 2002; Bell et al., 2007; Gómez and Espadaler, 2007; Krantz and Walter, 2009). This classification allowed us to consider the most important trophic guilds in soils food webs, and to relate *a priori* classification with stable isotope signatures of soil, animals, and plants. Detritivores were defined *sensu lato*, because despite most species are expected to feed on dead organic matter, some species may also feed on living components of organic matter not analysed here. This might be especially true for some

Staphylinid beetles, which may feed on dead arthropods and dung, but also on fungal hyphae (Thayer, 2016).

We consequently pooled together the treatments showing no differences between them in baseline-corrected  $\delta$ -values. For this new combination of treatments, we repeated the former statistical test twice, once for the analysed species present across and therefore shared between treatments (*common* species), and once for the analysed species present in a single treatment (*unique* species). We proceeded in that way because *common* species are likely to forage across the studied area, therefore integrating the environmental isotopic variability, whereas *unique* species are likely to have limited foraging ranges, therefore reflecting the isotopic signature characteristic of a sample. These analyses assured that for *common* species the isotopic baseline of multiple treatments is used and that all the isotopic variability from the samples of the study area in which the species is present is taken into account.

### 2.4.3. Food web structure

To delimit food webs across space, we followed the foundations of delimiting ecological systems of Post et al. (2007). That is, we considered macroinvertebrates of two treatments to constitute different food webs if they showed differences in community composition and stable isotope signatures. For characterizing each web, we built ellipses of isotopically similar macroinvertebrates (trophic groups) by conducting pairwise comparisons between species' isotopic niches ( $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ ) within each trophic guild. We included in this analysis only those species with at least four individuals to ensure the robustness of the test; the remaining species were assigned to the closest trophic group, and we adjusted the level of significance by setting a conservative threshold of  $P < 0.01$ . The number of estimated trophic levels (TL) for each food web was calculated as  $\text{TL} = 2 + (\delta^{15}\text{N}_{\text{top predator}} - \delta^{15}\text{N}_{\text{baseline}}) / \Delta\delta^{15}\text{N}$  (Post, 2002), where 2 is the TL of the primary consumer used to estimate the baseline ( $\delta^{15}\text{N}_{\text{baseline}}$ ),  $\delta^{15}\text{N}_{\text{top predator}}$  is the  $\delta^{15}\text{N}$  of the top predator in the food web, and  $\Delta\delta^{15}\text{N}$  is the increase in  $\delta^{15}\text{N}$  per trophic level (3.4‰; McCutchan et al., 2003; Potapov et al., 2019).

Analyses were run with the software PERMANOVA + for PRIMER v. 6 (Anderson et al., 2008) and with the package vegan (Oksanen et al., 2019) in R environment (R Core Team, 2019).

## 3. Results

### 3.1. Plant community composition

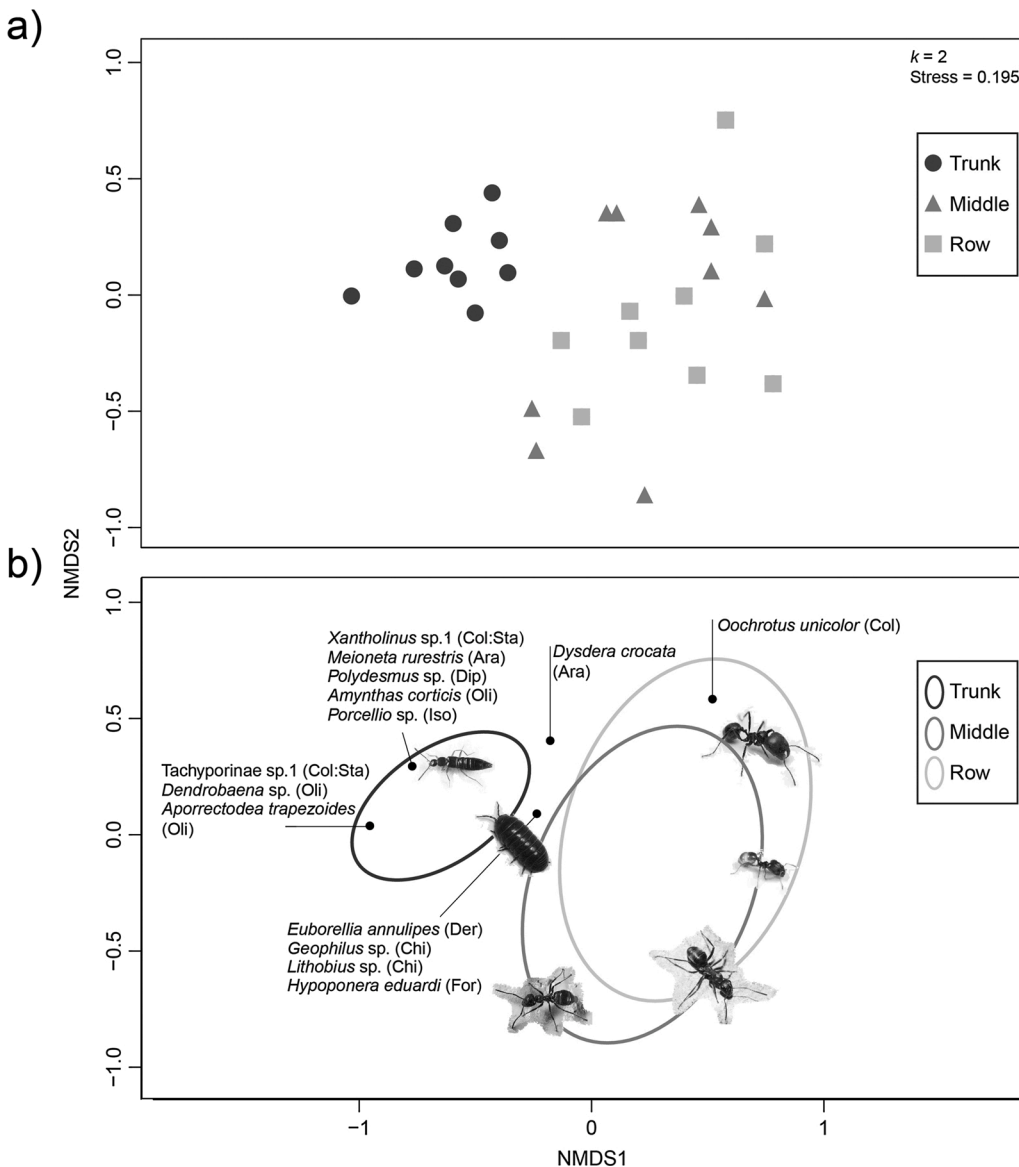
We collected a total of 27 species of plants from 17 families (Table A.1). Plant community changed across treatments (pseudo- $F_{2,16} = 6.14$ ,  $P < 0.001$ ). Trunk communities were different from those of Middle and Row samples ( $t = 2.64$ ,  $P = 0.0059$ ;  $t = 2.84$ ,  $P = 0.0024$ ; respectively), but there were no differences between Middle and Row ( $t = 1.18$ ,  $P = 0.23$ ).

The vegetation of Trunk samples was dominated by the nitrophilous *Parietaria officinalis* (Urticaceae) (60 % of all biomass), whereas Middle and Row were dominated by several grass species (76 %), mainly *Avena barbata*, *Hordeum murinum*, and *Cynodon dactylon* (the last one is  $C_4$ ). No differences in plant biomass between treatments were found (pseudo- $F_{2,16} = 0.74$ ,  $P = 0.49$ ).

### 3.2. Macroinvertebrate community composition

We collected a total of 3838 macroinvertebrate individuals belonging to 82 species and 36 taxonomic levels (family or higher) (Table A.2).

The macroinvertebrate community also changed across treatments (pseudo- $F_{2,16} = 5.48$ ,  $P < 0.001$ , Fig. 1a). As in plants, Trunk communities were different from those of Middle and Row samples ( $t = 2.65$ ,  $P = 0.0014$ ;  $t = 2.75$ ,  $P = 0.0012$ ; respectively), with only marginally significant differences between Middle and Row ( $t = 1.48$ ,  $P = 0.053$ ) (Fig. 1a).



**Fig. 1.** (a) Non-metric multidimensional scaling (NMDS) of the macroinvertebrate community composition at three increasing distances from the citrus trees (Trunk, 20 cm; Middle, 150 cm; Row, 350 cm) ( $N = 9$  per treatment). (b) Interpretation in terms of taxa of the NMDS diagram in (a). For each treatment, the ellipse encompasses all samples. Species with at least 5% contribution to the dissimilarity among treatments (SIMPER analyses,  $P < 0.05$ ) are sketched (not to scale; clockwise direction: *Messor barbarus*, *Pheidole pallidula*, *Formica rufibarbis*, *Lasius grandis*, *Armadillidium vulgare*, and *Aleocharinae*). Other species contributing less to dissimilarity (amounting up to ~95 % total dissimilarity between treatments) are indicated in the NMDS region in which they exert influence (all species in a list connected to a point are approximately around this point). Taxonomic groups are indicated in parenthesis (see Fig. 2 for details). Stress (goodness of fit) and  $k$  dimensions (axes of the best ordination fit) based on Bray-Curtis dissimilarity are provided in the top right corner.

The macroinvertebrate community composition was weakly correlated with the plant community composition (Mantel test,  $r = 0.18$ ,  $P = 0.006$ ).

The macroinvertebrate community of Trunk samples was characterized by Aleocharinae staphylinid beetles and the woodlice *Armadillidium vulgare* (Fig. 1b). Other species of staphylinid beetles (*Xantholinus* sp. and *Tachyporinae*), woodlice (*Porcellio* sp.), the spiders *Dysdera crocata* and *Meioneta rurestris*, the centipedes *Geophilus* sp. and *Lithobius* sp., the earwig *Euborellia annulipes*, the millipede *Polydesmus* sp., and earthworms (*Amyntas corticis*, *Dendrobaena* sp., and *Aporrectodea trapezoides*) were also characteristic of Trunk samples (Fig. 1b). The macroinvertebrate community of Middle and Row samples was characterized mostly by the presence of several ant species (*Messor barbarus*, *Pheidole pallidula*, *Formica rufibarbis*, *Lasius grandis*, and *Hypoponera eduardi*) and the beetle *Oochrotus unicolor*, which is indeed a myrmecophilous beetle associated to *M. barbarus* (Bernard, 1967) (Fig. 1b).

Trunk samples had higher abundances of staphylinid beetles (Coleoptera: Staphylinidae), woodlice (Isopoda), centipedes (Chilopoda) and millipedes (Diplopoda) (Fig. 2). Earthworms (Oligochaeta) and snails (Gastropoda) were also relatively more abundant in Trunk samples. Conversely, ants were very abundant in Middle and Row samples (80.2

% of total individuals, vs 24.6 % in Trunk). Erythraeidae mites (Acari; 1–3 mm body width) and cockroaches (Blattodea) were also more abundant in Middle and Row samples. Excluding ants, total macroinvertebrate abundance was higher in Trunk samples ( $1666 \pm 260$  individuals  $m^{-2}$ ) than in Middle and Row samples ( $376 \pm 46$  individuals  $m^{-2}$ ) (Fig. 2).

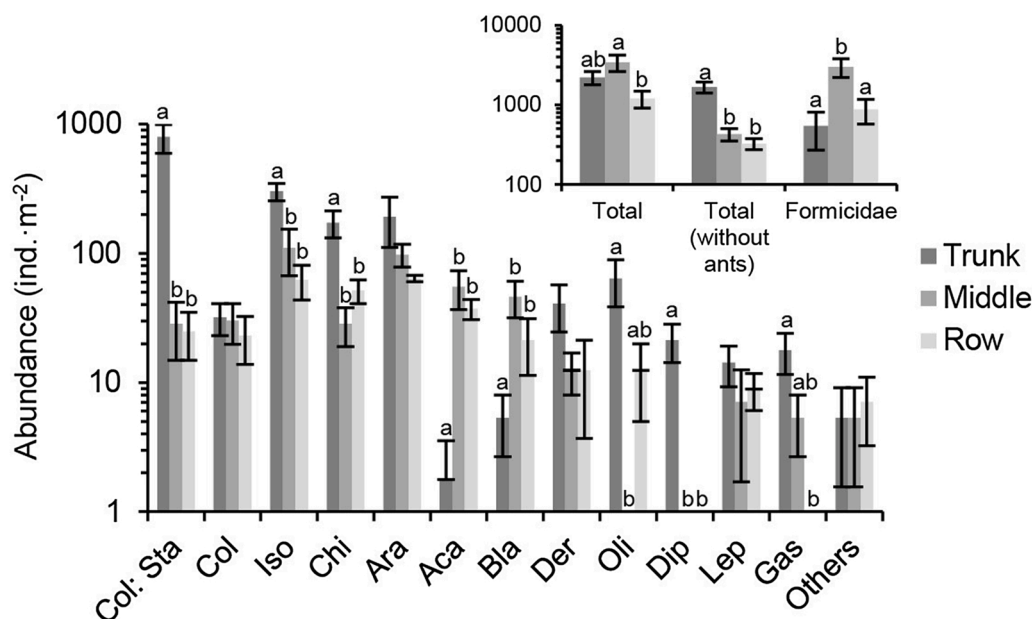
### 3.3. Stable isotope signatures of soil, plants, and macroinvertebrates

There were no differences in soil  $\delta^{13}C$  among treatments (pseudo- $F_{2,16} = 2.03$ ,  $P = 0.16$ ), but  $\delta^{15}N$  decreased at increasing distances from the tree trunk (pseudo- $F_{2,16} = 104.53$ ,  $P < 0.001$ ) (Fig. 3a, b).

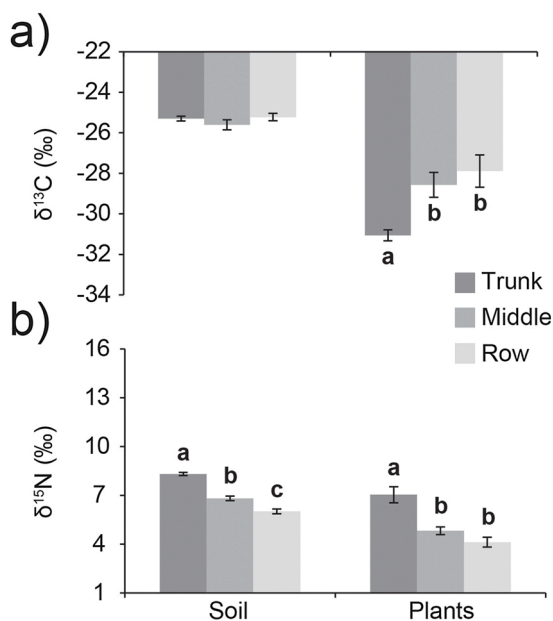
Soil organic matter content (%) varied similarly, being  $7.7 \pm 0.6$ ,  $4.3 \pm 0.6$ , and  $3.6 \pm 0.2$  in Trunk, Middle, and Row samples, respectively ( $N = 4$  per treatment).

Plants had different  $\delta^{13}C$  and  $\delta^{15}N$  values among treatments (pseudo- $F_{2,43} = 14.19$ ,  $P < 0.001$ ; pseudo- $F_{2,43} = 7.88$ ,  $P = 0.0015$ ; respectively). Trunk plants had the lowest  $\delta^{13}C$  and the highest  $\delta^{15}N$  levels, but no differences in isotopic signatures were found between Middle and Row plants (Fig. 3a, b).

Table A.3 contains the complete dataset of stable isotope signatures for soil and plants.



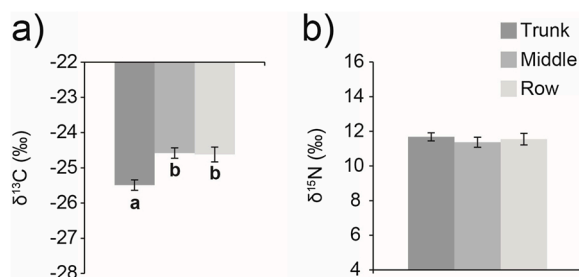
**Fig. 2.** Macroinvertebrate abundance (individuals·m<sup>-2</sup>) (mean ± SE) at three increasing distances from the citrus trees (Trunk, 20 cm; Middle, 150 cm; Row, 350 cm) (N = 9 per treatment). Key: Col:Sta, Coleoptera: Staphylinidae; Col, Coleoptera (other groups); Iso, Isopoda; Chi, Chilopoda; Ara, Araneae; Aca, Acari (1-3 mm body width); Bla, Blattodea; Der, Dermaptera; Oli, Oligochaeta; Dip, Diplopoda; Lep, Lepidoptera; Gas, Gastropoda. Different letters indicate significant differences between treatments (P < 0.05). Note the log scale used in the y-axis.



**Fig. 3.** (a) δ<sup>13</sup>C and (b) δ<sup>15</sup>N values (mean ± SE) of soil and plants at three increasing distances from the citrus trees (Trunk, 20 cm; Middle, 150 cm; Row, 350 cm). Different letters indicate significant differences between treatments (P < 0.05).

Macroinvertebrate isotopic signatures were corrected by their isotopic baselines (soil δ<sup>13</sup>C and δ<sup>15</sup>N as covariates). Macroinvertebrates had different δ<sup>13</sup>C among treatments (pseudo-F<sub>2,207</sub> = 7.25; P < 0.001). Trunk macroinvertebrates were depleted in <sup>13</sup>C with respect to Middle and Row communities (t = 3.11, P = 0.002; t = 2.17, P = 0.034, respectively), but there were no differences between Middle and Row macroinvertebrates (t = 0.61, P = 0.56) (Fig. 4a). In contrast, no differences in δ<sup>15</sup>N were found among treatments (pseudo-F<sub>2,207</sub> = 0.22, P = 0.81) (Fig. 4b).

We subsequently pooled together Middle and Row samples (Middle + Row thereafter). Twenty-six per cent of the analysed macroinvertebrate species (representing 31 % of the abundance) were common to Trunk and Middle + Row samples (Fig. A.2). In the common species,



**Fig. 4.** (a) δ<sup>13</sup>C and (b) δ<sup>15</sup>N values (mean ± SE) of macroinvertebrates at three increasing distances from the citrus trees (Trunk, 20 cm; Middle, 150 cm; Row, 350 cm). Soil isotopic δ<sup>13</sup>C and δ<sup>15</sup>N is used as the isotopic baseline, and therefore included in the models as covariates. Different letters indicate significant differences between treatments (P < 0.05).

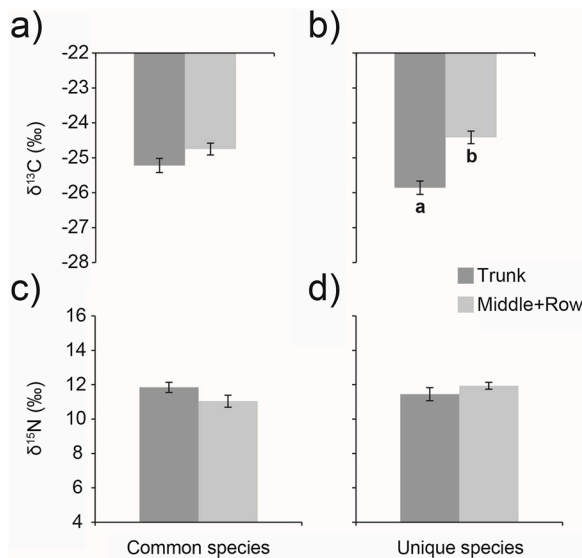
the observed differences in δ<sup>13</sup>C across treatments vanished (pseudo-F<sub>1,124</sub> = 2.13, P = 0.14) (Fig. 5a). In contrast, the unique species to Trunk sites maintained the isotopic differences in δ<sup>13</sup>C in comparison to unique species to Middle + Row samples (pseudo-F<sub>1,75</sub> = 9.11, P = 0.02) (Fig. 5b), as in the entire macroinvertebrate community. No differences in δ<sup>15</sup>N were found, neither for the common species (pseudo-F<sub>1,124</sub> = 3.70, P = 0.057) nor for the unique species (pseudo-F<sub>1,75</sub> = 0.43, P = 0.51) (Fig. 5c, d). Excluding ants from the analysis (unique to Middle + Row samples) yielded identical results.

Table A.4 contain the complete dataset of stable isotope signatures for macroinvertebrates.

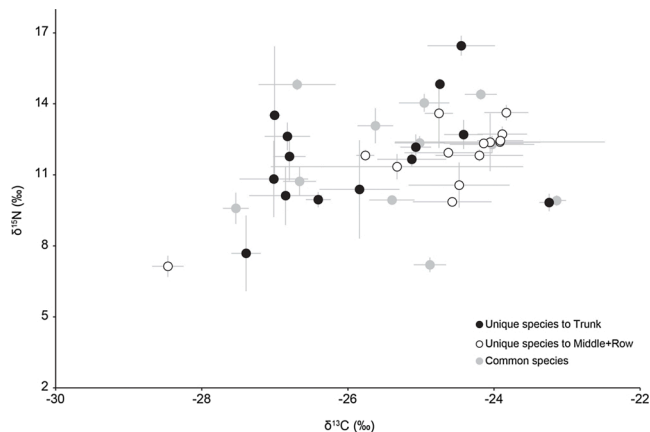
### 3.4. Food web structure

As the Middle and Row macroinvertebrate communities were similar in species composition and isotopic signatures, and both differed from the communities of the Trunk samples, we delimited two food webs: one for Trunk, at 20 cm from the tree trunks, and one for Middle + Row, at 150/350 cm.

There was a clear spatial structure in macroinvertebrate soil food webs at one-meter scale, with distinct species near the tree trunks showing different isotopic signatures than those species located farther away (Fig. 6). These food webs, though, were partially connected by common species (26 % of total species), which showed no differences in



**Fig. 5.** (a, b)  $\delta^{13}\text{C}$  and (c, d)  $\delta^{15}\text{N}$  values (mean  $\pm$  SE) of (a, c) *common* species (present across treatments) and (b, d) *unique* species (present in a single treatment) of macroinvertebrates between Trunk (20 cm from the citrus trees) and Middle + Row (onwards). Soil isotopic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is used as the isotopic baseline, and therefore included in the models as covariates. Different letters indicate significant differences between treatments ( $P < 0.05$ ).



**Fig. 6.** The spatial structure of soil food webs at one-meter scale. The Trunk food web (20 cm distant from the citrus trees) had distinct macroinvertebrate communities with different isotopic signatures (solid black circles) than the Middle + Row food webs (150/350 cm distant) (open black circles). Both food webs were partially connected by common species (26 % of total species) (gray circles) showing no differences in isotopic signatures between food webs.

isotopic signatures between food webs (Fig. 6).

In all food webs, the vast majority of  $\delta^{13}\text{C}$  signatures of macroinvertebrates were similar to those of soil (Fig. 7a, c), but in Trunk food web there was a large group of species with lower  $\delta^{13}\text{C}$  values than the soil (Fig. 7a). As a whole, macroinvertebrates spanned more than 4 trophic levels both in the Trunk and in the Middle + Row webs (Fig. 7b, d).

We established nine trophic groups in Trunk food web and eight trophic groups in Middle + Row food web based on the isotopic signature of the macroinvertebrate species (Table A.5). The Trunk food web (Fig. 7b) consisted of five trophic groups of detritivores, one group of herbivores, one group of omnivores, and two groups of predators. The Middle + Row food web (Fig. 7d) had two trophic groups less of detritivores and one more of predators than the Trunk food web.

No difference was found in the  $\delta^{13}\text{C}$  signature among trophic groups,

whereas there was a gradual pattern of increase in  $\delta^{15}\text{N}$  from detritivores to predators, with overlap among trophic guilds (Fig. A.3).

#### 4. Discussion

The composition and trophic structure of the macroinvertebrate community of the citrus grove varied spatially, as indicated by the presence of different species assemblages and shifts in isotopic signatures. We were able to distinguish two partially compartmentalized food webs in the study area, which differed in the abundance and identity of detritivores and predators as well as the potential sources of energy used. Our results showed that even within a single ecosystem, at scales as small as one meter, there can exist consistent differences in food web composition and structure. The most parsimonious ecological explanation behind the observed pattern is that spatial variability in organic matter content and water availability drives changes in community composition (both in abundance and identity) and trophic structure (energy pathways and functional groups).

##### 4.1. Spatial variability in environmental factors

Fertilization with organic manure beneath the citrus canopies has two notable effects. First, the soil content of organic matter was higher there and decreased outwards. Second, as the fertilizer was enriched in  $^{15}\text{N}$  compared to the soil (Fig. 7a), values of  $\delta^{15}\text{N}$  in the soil display the same pattern as that of the organic matter content.

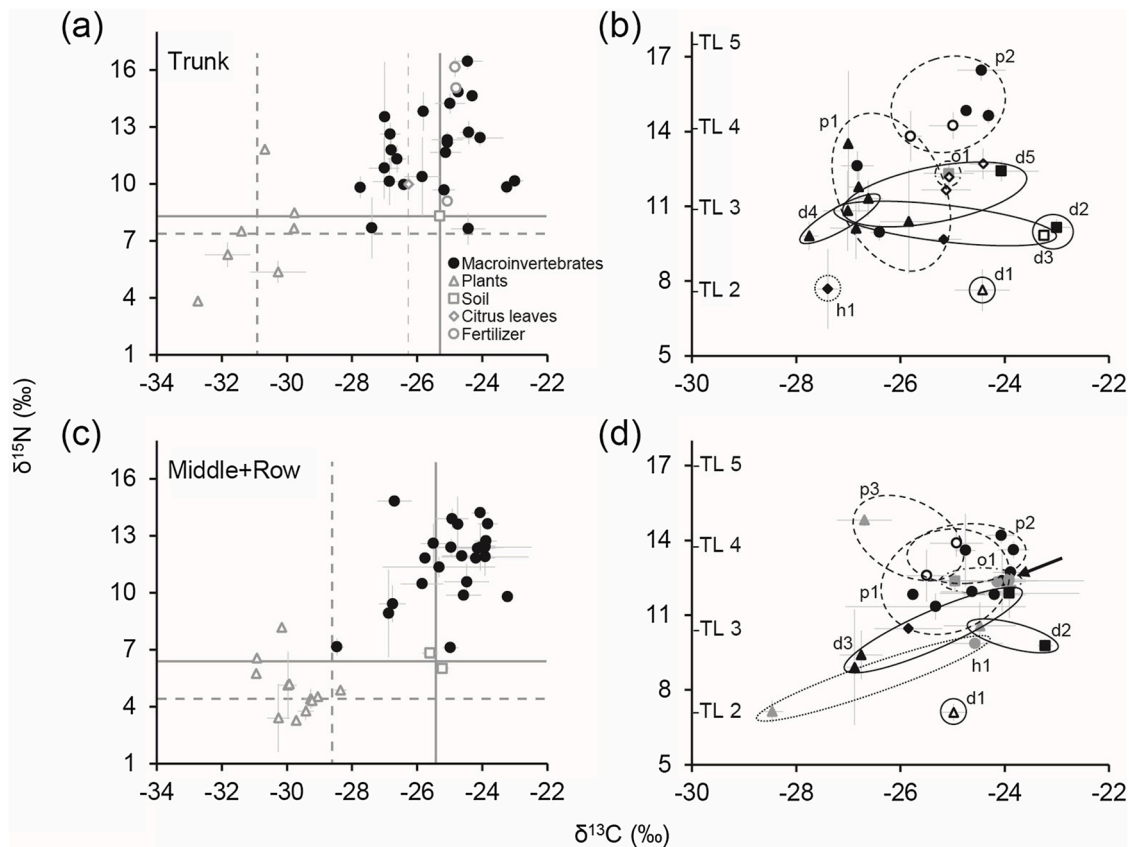
Signatures of  $\delta^{15}\text{N}$  in plants are assumed to reflect those of bulk soil (Marshall et al., 2007) and that is what we found. Plants near the tree trunks had lower  $\delta^{13}\text{C}$  values than plants from the other treatments. This was likely due to the effect of irrigation, as water-stressed plants are generally enriched in  $^{13}\text{C}$  because of an increase in water-use efficiency (Marshall et al., 2007). The difference in  $\delta^{13}\text{C}$  between Trunk and Middle/Row plant communities was further broadened by the presence in the latter of *Cynodon dactylon*, a  $\text{C}_4$  grass, since  $\text{C}_4$  plants present higher  $\delta^{13}\text{C}$  values than  $\text{C}_3$  plants ( $\sim -14\text{‰}$  versus  $\sim -27\text{‰}$ , respectively, Marshall et al., 2007) (see  $\delta$ -values weighted by biomass with and without  $\text{C}_4$  plants in Table A.3).

In an agricultural semiarid ecosystem, organic matter and water are expected to be the main limiting factors for soil macrofauna, and therefore the main drivers of small-scale spatial variability in soil food webs. Other soil properties such as structure, porosity and pH, among others, are also important in determining soil communities (van Straalen and Verhoef, 1997v; Bardgett, 2005) but probably play a secondary role here. They might be important in ecosystems where other processes, such as animal tramping or local concentration of pesticides, generate spatial variability in physicochemical soil properties, eventually leading to spatial variability in soil food webs.

##### 4.2. Spatial variability in macroinvertebrate community composition

The macroinvertebrate community was significantly related to the plant community, but the correlation was low. This result does not imply causality but simply shows that macroinvertebrates and plants experience a common spatial variability in environmental conditions.

The macroinvertebrate community beneath the canopy differed from that located farther away, mainly in having more detritivores (Aleocharinae staphylinid beetles, woodlice, and earthworms) and predators (centipedes). This is likely linked to the fact that decomposer communities are most developed in organic soils (Anderson, 1975; Bengtsson et al., 1998), and benefit from organic farming. Thus, the organic fertilizer applied beneath the tree canopies may benefit faunal decomposition and predation via a bottom-up effect (Birkhofer et al., 2008). Hygrophilous millipedes (also detritivorous) and herbivorous snails were also more abundant near the tree trunks. During dry periods, water availability in irrigated areas improves resource quality (plants) and therefore boosts herbivore populations (Pérez-Fuertes et al., 2015).



**Fig. 7.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  SE) values of macroinvertebrates, plants, soil, and other environmental items for the (a) Trunk (20 cm distant from the citrus trees) and (c) Middle + Row (150/350 cm distant) food webs. Vertical (for mean  $\delta^{13}\text{C}$ -values) and horizontal (for mean  $\delta^{15}\text{N}$ -values) lines are plotted for soil (solid) and plant (dashed) signatures. Note the thinner vertical dashed line for leaves of citrus trees. (b, d) The same food webs, with macroinvertebrates of a given trophic guild regrouped into ellipses of isotopically similar species, representing trophic groups of detritivores (solid line), herbivores (dotted line), omnivores (dot-dash line), and predators (dashed line). Each trophic group is labelled (e.g., d1 = trophic group 1 of detritivores).  $\delta^{15}\text{N}$  value for each trophic level (TL) is indicated on the y-axis. Key for graphics (b) and (d): solid black circle, Araneae; solid black square, Isopoda; solid black triangle, Staphylinidae; solid black diamond, Gastropoda; open black circle, Chilopoda; open black square, Diplopoda; open black triangle, Blattodea; open black diamond, Oligochaeta; solid grey circle, Formicidae; solid grey square, Dermaptera; solid grey triangle, Coleoptera; Acari (1-3 mm body width) not visible, pointed with an arrow.

Conversely, ants, which are typically found in relatively dry environments (Hölldobler and Wilson, 1990), were more abundant farther away.

#### 4.3. Stable isotope signatures of macroinvertebrates

The isotopic composition of the environment affects the isotopic composition at the base of the food web, which in turn affects the entire community. Therefore, when comparing food webs, it is necessary to distinguish between differences in isotopic signatures due to shifts in trophic structure from those due to changes in the isotopic baseline itself (Post, 2002). While the isotope signatures of less-mobile organisms may closely reflect those of their immediate environment, an increase in the size of an organism's foraging area is expected to lead to the buffering of some of the environmental isotopic variability (Bearhop et al., 2004; Brewitt et al., 2015).

By correcting isotopic signatures of macroinvertebrates by their isotopic baseline, we were able to determine that macroinvertebrates near the tree trunks were depleted in  $^{13}\text{C}$  in comparison to those located farther away. This difference was mediated by *unique* species, that is, species only present near the trunk. In contrast, species foraging across the studied area integrated the spatial isotopic variability. This suggests that changes in macroinvertebrate community composition (mainly through the addition of new species) near the tree trunk drive the exploitation of an energy channel which is not exploited by the more distant organisms. We hypothesize that nutrient and water subsidies

near the tree trunk enhance plant quality, fostering the exploitation of the grazing energy channel. In turn, beneath the canopy, the presence of citrus leaves and the tree root system may foster the consumption of other food components not analysed here, such as decomposed plant material, root exudates and mycorrhizal fungi, which have higher  $\delta^{13}\text{C}$  signatures than fresh plant material (Hobbie et al., 2001; Boström et al., 2008; Pollierer et al., 2009) and are exploited by multiple soil organisms (Chahartaghi et al., 2005; Klärner et al., 2013).

The analysis of bulk stable isotopes of soil animals provides information about potential food resources, that is, we can infer their diet based on the isotopic composition of the other components of the food web (see the *isotopic map* in Potapov et al., 2019). Whilst the interpretation of the stable isotopic values of macroinvertebrates near the tree trunk is consistent with our hypotheses (*i.e.*, they might be feeding on plants or fungi), a definitive proof would require advanced techniques such as isotope labelling or analyses of compound-specific stable isotopes of fatty acids (Pollierer et al., 2007, 2012).

#### 4.4. Food webs in space: delimitation and main characteristics

We defined two food webs at the study site. The composition of the macroinvertebrate community near the tree trunks was different from that located above one meter away, largely because of a higher abundance of detritivores and predators. In addition, Trunk communities seem to rely more on plant-based resources, as demonstrated by lower baseline-corrected  $\delta^{13}\text{C}$ -values in the macroinvertebrate community.

The two food webs were thus compartmentalized, albeit partially connected by the 26 % of total species *common* to both communities (representing 31 % of the abundance).

The similarity in  $\delta^{13}\text{C}$  values between trophic guilds and their close relation to the isotopic soil signature in the two food webs suggests the presence of a generalized pathway of energy flux from soil organic matter to detritivores to predators (e.g., Halaj et al., 2005). Enrichment in  $^{13}\text{C}$  relative to plant material is a typical feature in soil food webs (also known as “detrital shift”) and is thought to be related to the acquisition of  $^{13}\text{C}$ -enriched microbial biomass (saprotrophic fungi and bacteria) from soil organic matter (Potapov et al., 2019). However, the wide range of  $\delta^{13}\text{C}$  ( $5.0\text{‰} \pm 0.2$  for the two food webs) entails greater compartmentalization stemming from multiple potential pathways between primary production and processed soil organic matter (Schmidt et al., 2004; Pollierer et al., 2009).

In contrast, there was a pattern of  $^{15}\text{N}$  enrichment from detritivores to predators. This means that classifying macroinvertebrates into *a priori* trophic guilds is useful for understanding food web structure. However, there was a large degree of intraguild variability, as reflected by the  $\delta^{15}\text{N}$  range in detritivores ( $4.9 \pm 0.1\text{‰}$ ) and predators ( $5.6 \pm 1.2\text{‰}$ ). Thus, such classification is only useful when the taxonomic resolution is high (e.g., Mestre et al., 2013). The high intraguild variability and the overlap between trophic guilds corroborates the idea that soil food webs are composed of a trophic continuum rather than of well-defined trophic levels (Scheu and Falca, 2000; Ponsard and Arditi, 2000), and contain functional groups within trophic guilds (Oelbermann and Scheu, 2010).

#### 4.5. Food webs in space: trophic groups of detritivores and predators

*Loboptera decipiens* (Blattodea) appears to be the primary decomposer at the base of both food webs (d1; Fig. 7b and d), with isotopic values like those of soil. Instead, the remaining detritivores have higher  $\delta^{15}\text{N}$  -values, seeming to act as secondary decomposers, that is, feeding on microbially processed organic matter (d2–d5; Fig. 7b and d). Among these, staphylinid beetles had the lowest  $\delta^{13}\text{C}$  values (below soil  $\delta^{13}\text{C}$ ) (d3-d4, Fig. 7b, d), suggesting that they may rely on mycorrhizal fungi, which are enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to fresh plant material (Hobbie et al., 2001). While in the Trunk food web this distinctive energy channel is well developed (Fig. 7b), in Middle + Row food web it is marginal (Fig. 7d). The remaining detritivores had higher  $\delta^{13}\text{C}$  values and were mainly represented by millipedes and earthworms (only in Trunk food web; d3 and d5, respectively, Fig. 7b) and isopods (d2, d5, Fig. 7b; d2, d3, Fig. 7d), potentially feeding on humified soil organic matter and  $^{15}\text{N}$ -enriched detritus such as faecal material, which is also enriched in  $^{13}\text{C}$  through microbial processing (DeNiro and Epstein, 1978).  $^{13}\text{C}$  enrichment in millipedes and woodlice can also be related to the presence of inorganic carbon in their cuticle (Maraun et al., 2011; Potapov et al., 2019).

There were two distinct groups of predators in the Trunk food web (Fig. 7b). The first group (p1) was composed of predatory staphylinid beetles and lynphiid spiders (average body length 2 mm; Nentwig et al., 2016), potentially exploiting the distinctive energy channel preying upon other staphylinids. The second group (p2) is formed of chilopods and bigger spiders (average body length 10 mm; Nentwig et al., 2016), potentially preying on secondary decomposers below them. Since the predator group p2 is one trophic level above p1, intraguild predation must also be considered (McNabb et al., 2001; Mestre et al., 2013), with the potential for intragroup predation due to the presence of the spider *Nemesia* sp. (the top predator, TL 4–5). In the Trunk food web, then, intraguild predation might be restricted to bigger species and may be less frequent due to high prey availability (Wise et al., 1999; Birkhofer et al., 2011). However, there is evidence that intraguild predation may be a pervasive feature of the Middle + Row food web (p1-p3; Fig. 7d). Although prey subsidies from Trunk may enable the existence of a diversified predator community (Polis et al., 1997), there is a generalized scarcity of potential food sources other than ants, as well as a high

overlap between trophic groups (p1-p2) and trophic niches of species (mainly spiders and chilopods). Besides, the mean  $^{15}\text{N}$  enrichment in predators is 3.1‰ that of detritivores (versus 2‰ in Trunk).

#### 4.6. Concluding remarks

In the studied agroecosystem, the gradient in soil organic matter content (from organic fertilizer) and water availability (from irrigation) from the tree trunks outwards was reflected in the isotopic signatures of both soil and plants. This spatial variability in environmental factors is thought to be the main driver of changes in food web structure. These subsidies of detritus and water may benefit decomposers and predators via a bottom-up effect and also through the exploitation of plant-based resources near the tree trunks, resulting in the creation of a food web that is distinct and predominantly independent from that located just one meter away.

Thus, the present study shows that

- spatial variability in macroinvertebrate soil food webs can be present at a scale as small as one meter if spatial variability in environmental factors is high enough, and
- within-habitat heterogeneity should be taken into account when determining sampling scales and food-web-related properties and ecosystem processes.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data is available upon request.

#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.pedobi.2021.150751>.

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