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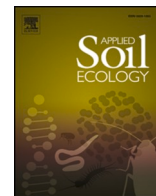
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Indirect effect of earthworms on wheat-aphid interactions

Thomas Damestoy^{*}, David Houben, Léa Kervroëdan, Laurine Ambeza, Emma Pluchard, Anne-Maïmiti Dulaurent

UniLaSalle, AGHYLE, UP.2018.C101, FR-60026 Beauvais, France

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ABSTRACT

Studies on interactions between plants and belowground organisms, or between plants and aboveground organisms, are numerous and highlight their importance in understanding ecosystem functioning. However, these two compartments are rarely studied together, despite the direct link formed by the plant itself. Soil organisms can influence interactions between plants and herbivores by changes in plant growth and chemistry. Under controlled conditions the influence of earthworms and aphids on wheat was investigated. The effect of earthworms on aphid reproduction was also investigated, to demonstrate the indirect plant-trait-mediated effects. We showed a significant effect of both above and belowground organisms on wheat. Interestingly, nitrogen content was higher in the presence of aphids ($1.63 \pm 0.07\%$) or earthworms alone ($1.58 \pm 0.04\%$) and even higher in presence of both ($2.07 \pm 0.07\%$). Moreover, we showed a strong effect of earthworms on aphid reproduction rates which are almost twice as high as in the absence of earthworms where we observed a reproduction rate of 32 individuals per day compared with 57 in the presence of earthworms. The presence of aphids led to a 16 % reduction in above-ground biomass compared with the control (1.83 ± 0.08 g vs 2.19 ± 0.09 g for the control), while the presence of earthworms led to an 46 % increase (3.20 ± 0.12 g vs 2.19 ± 0.09 g), and the presence of both organisms had no significant effect on the above-ground biomass of wheat compared with the control (2.39 ± 0.08 g vs 2.19 ± 0.09 g). These results could have implications for pest management, as they indicate that the presence of earthworms compensate the negative effect of aphids on plant biomass, probably by acting on nutrient uptake and plant defensive traits. By addressing the effects of earthworms on both the pest performances and plant traits, we highlight the need for further research on the indirect effects of below-ground compartments on pest performance. This study builds towards a better understanding of the interaction between soil, plant and pests and highlights the importance to study the pest management with a holistic view of agroecosystems.

1. Introduction

Interactions between aboveground and belowground organisms are key drivers of plant growth and ecosystem functioning (De Deyn and Van der Putten, 2005). By occupying two connected compartments, plants facilitate the interactions between air and soil (van Geem et al., 2013). However, most studies focus on the interactions between either roots and belowground organisms or shoots and aboveground organisms even though that both compartments are generally considered as interconnected parts of ecosystems (Heinen et al., 2018; Ke and Scheu, 2008; Singh et al., 2014; Wardle et al., 2004; Wurst, 2010). Potential feedback between below and aboveground organisms, which are linked by their dependence on the same plant, should be better investigated to gain new insights into the functioning of agroecosystems and their role

in pest control and sustaining food production.

Soil organisms affect plant performance either directly through trophic links at the root level (e.g. herbivory, symbiosis), or indirectly by modulating the physical and chemical properties of the soil (Brown et al., 2004; Edwards, 2004) but also by modifying the soil microbiome (Blouin et al., 2005; Hodson et al., 2023; Medina-Sauza et al., 2019). In this way, the activity of belowground organisms could have a cascading effect on the performance of aboveground herbivores by acting on the plant traits, including plant biomass or plant chemistry (Bezemer and Vandam, 2005; Puga-Freitas and Blouin, 2015; Scheu, 2003; Wurst, 2010). Among soil organisms, earthworms are extremely important for soil functioning (Bertrand et al., 2015; Blouin et al., 2013; Cunha et al., 2016; Edwards, 2004) and plant growth (Brown et al., 2004; Scheu, 2003; van Groenigen et al., 2014; Xiao et al., 2018). Due to their large

^{*} Corresponding author.

E-mail address: damestoythomas@hotmail.fr (T. Damestoy).

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body size, high consumption rates and burrowing activity, they play a key role in incorporating organic matter and making nutrients available for plants (Edwards, 2004; Edwards and Arancon, 2022a, 2022b). As a result, plant performance may be stimulated by earthworms (Ke and Scheu, 2008; Poveda et al., 2005; Scheu, 2003) which in turn might affect the food quantity and quality for herbivores (Xiao et al., 2019). The consequences and the magnitude of these changes on herbivore performance are however not fully elucidated and seem to be dependent on various factors such as herbivore feeding guild and plant traits. For instance, the meta-analysis by Xiao et al. (2018) reveals that earthworm presence increases by 20 % plant growth and by 11 % nitrogen content which has neutral effects on plant resistance against chewing herbivores (e.g. caterpillars), negative effects on plant resistance against phloem-feeding herbivores (e.g. aphids) but positive effects on plant resistance against cell-feeders (e.g. thrips).

Among the most problematic pest insects, aphids are r-strategists with an asexual reproduction by parthenogenesis giving them the ability to rapidly reach high population densities. In addition, when the host quality declines, aphids can produce winged morph to facilitate dispersal to new plants (Goggin, 2007). These factors, along with their ability to colonize virtually every part of the plant, to consume large quantities of photo-assimilates, to manipulate plant responses, to be the vector of nearly 30 % of the plant viruses described and to resist many insecticides, make them highly problematic pests (Brault et al., 2010; Giordanengo et al., 2010; Larson Vasquez, 1995). They feed on phloem sap, a high-quality diet due to its high sugar concentrations providing an abundant source of carbon and energy, and nitrogen predominantly in the form of free amino acids. In addition, phloem sap is generally free of toxins and feeding deterrents (Douglas, 2006). Moreover, plant nitrogen content is a key plant trait in governing the performance of herbivores. As an important limiting element for herbivore insects (Hansen et al., 2020; Mattson, 1980; Newington et al., 2004), low nitrogen content (or high C:N ratio) is generally associated with a low nutritive quality, reducing the performance of herbivore insects, which tend to prefer nitrogen-rich tissues (Haase et al., 2008; Mattson, 1980; Schütz et al., 2008; White, 1984). The strongest evidence for the limitation of herbivore performance by plant nitrogen comes from experiments where plants were fertilized with mineral nitrogen. For example, Aqueel and Leather (2011) showed an increase in aphid, *Sitobion avenae* (Fabricius, 1794) performance on wheat with nitrogen fertilization. Potentially as increase in nitrogen assimilation by plants induced by earthworm activity could lead to an increase in the herbivore densities, most of which are limited by the nitrogen content of their tissues (Mattson, 1980; White, 1984; Wurst and Jones, 2003).

The lack of integration of studies on the effects of soil organisms on plant performance and on the aboveground system opens a large scope for future research in pest management (Heinen et al., 2018; Puga-Freitas and Blouin, 2015; Van der Putten et al., 2001; Wurst, 2010; Wurst and Jones, 2003). Furthermore, with the tightening of legislation on chemical crop protection, it is imperative to better understand the interactions within the crop and to have a more holistic view of the agroecosystem and pest management. In the present study, the direct influence of earthworms and aphids was investigated on root and shoot development and on the chemical composition of wheat under controlled conditions. The effect of earthworms on aphid reproduction was also investigated in order to elucidate the indirect plant-trait-mediated effects. This study hypothesized (i) a positive effect of earthworms on wheat biomass production and N content in wheat; (ii) a positive effect of earthworms on aphid reproduction rate by increasing nitrogen content in wheat; and (iii) that the negative effects of aphids are offset by earthworm activity. By doing so, this study builds towards a better understanding of the interaction between soil, plant and pest insects and highlight the importance to study the pest management with a more holistic view of agroecosystems.

2. Materials and methods

2.1. Study design

A factorial greenhouse experiment at the UniLaSalle Polytechnic Institute campus in Beauvais (Northern France, GPS: 49°27'58.0"N, 2°04'20.2"E) was performed using wheat under four conditions: absence/presence of earthworms, and absence/presence of aphids. A total of 60 pots (4 × 15 pots, height = 21.8 cm; diameter = 18.7 cm) was filled with soil. The study soil was a Haplic Luvisol and was sampled in an agricultural field. After being air-dried, the soil was sieved at 4 mm. Soil sieving at 4 mm removes stones, clods and trash while leaving microaggregates intact, ensuring therefore optimal soil respiration (Adekanmbi et al., 2020). Sieving at 4 mm for pot experiment facilitates root penetration and allows a better aeration as well as a better water infiltration into the pot, avoiding anaerobic conditions, especially for a silty soil. Gauze was attached at the bottom of each pot to prevent earthworms from escaping. On March 3, 2023, five untreated wheat seeds, *Triticum aestivum* L. (Fructidor variety) were planted. Plants were watered twice a week and kept in a greenhouse with ambient temperature (an average of 12 °C at night and 25 °C by day, for 61 days), humidity, and light. Once the seeds had germinated and reached the early tillering stage, the plants were thinned to two plants per pot.

On April 4, 2023, aphids and earthworms were inoculated. Fifteen pots were prepared for each treatment: 15 pots without earthworms nor aphids; 15 pots with aphids but without earthworms; 15 pots with earthworms but without aphids; 15 pots with both earthworms and aphids. Each pot was randomly distributed in the greenhouse. Two or three *Lumbricus terrestris* L. (*Lumbricidae*) were placed in each of the 15 aphid-free pots and the 15 pots with aphids. Similar earthworm densities have been reported for agroecosystems, but with considerable variation (Dulaurent et al., 2023), we therefore decided to work with similar biomasses in the pots corresponding to an average biomass of 9.37 ± 0.13 g (see Eisenhauer and Scheu, 2008). The earthworms were chosen so that their biomass was the same in each pot, ensuring that their activity would initially be similar. The presence of clitellum was not a targeted characteristic for this experiment. Earthworms were purchased at Decathlon® (Live fishing bait, Canadian worms). Six aphids of the species *Sitobion avenae* Fabricius (*Aphididae*), were placed on the plant leaves using a brush, in each of the 15 earthworm-free pots and the 15 pots with earthworms. Aphids reared on wheat were provided by Crisop society (Koppert Ervibank®). Nylon bags (100 × 50 cm) with 0.05 × 0.05 cm mesh (Kweekzak 100 × 50 cm from Vermandel Entomologie Speciaalzaak) were used to contain the aphids in the pots. To homogenize the experiment, nylon bags were placed around all the pots, with and without aphids, and all the pots were regularly randomly moved around the greenhouse.

At the end of the experiment (May 2, 2023), all the wheat was harvested, and the roots separated from the shoots. All shoot samples were left for 48 h at 8 °C to facilitate aphid counting to estimate a simplified reproduction rate (corresponding to the final number of aphids over the total number of days, 28 days). Aphids were counted, using mechanical counters on a clear, white surface. The roots were separated from the soil and then cleaned with clear water until no soil was visible on the roots to avoid any bias in the biomass and chemical analyses. Shoots and roots were dried for 48 h at 50 °C directly after aphid counting; weighted to obtain dry biomass; and finally, ground to fine powder before further chemical analyses. Shoot and root C and N contents were quantified with a gas chromatography in an automatic elemental analyser (FlashS-mart™; Thermo Fisher Scientific) using approximately 6.0 mg of dried powder. The accuracy of the measurement ranges varied for N from 0.01 % to 100 % and for C from 0.005 % to 100 %. Measurements taken on the sulfanilamide used for calibration and control had an accuracy of 0.36 % for nitrogen and 0.37 % for carbon.

2.2. Statistical analysis

The effect of the presence or absence of earthworms and aphids (two factors with 2 treatments; presence or absence of earthworms, and presence or absence of aphids) was tested on wheat traits (dry shoot and root biomasses; and C and N contents in shoots). The effect of the presence of earthworms was tested on aphid reproduction rate only for pots with aphids (*i.e.* $n = 30$). The model was run including N content as covariate in model testing the effect of the presence of earthworms on aphid reproduction rate. A sequential decomposition of variance was used to test the effects of each predictor. Shoot traits were fitted before the effect of the presence of earthworms. With this approach, if shoot traits (N content) mediate effects of earthworms on herbivore performance in this mechanistic model, then the significant main effects in the prior models (without covariates) should turn non-significant after including covariates.

For all models, Linear Models were used with a Gaussian error distribution (ANOVA and ANCOVA models). Log transformations of the response variable (N content in shoots) was used to satisfy the model assumptions. We then simplified the initial full model by sequentially removing terms with non-significant effects, starting with the least significant. We used Tukey test for pairwise comparison.

All analyses and figures were performed in R software, version 4.2.1 (R Core Team, 2022) with the packages: *car*, *multcomp*, *ggplot2*, *ggpubr* and *emmeans* (Fox and Weisberg, 2019; Hothorn et al., 2008; Kassambara, 2023; Lenth et al., 2024; Wickham, 2016). For more clarity in the Result part, data were expressed as *mean* \pm *standard error*.

3. Results

3.1. Effects of earthworms and wheat nitrogen content on aphid reproduction

In total, 180 aphids were applied on 30 pots (6 aphids per replicate). At the end of the experiment (28 days later) a total of 37,370 aphids were numbered. Furthermore, the presence of earthworms had a significant effect on aphid multiplication (Table 1), which was almost twice as high (Fig. 1). In the presence of earthworms, aphids multiplied at a mean rate of 57 individuals per day (± 3) whereas without earthworms, the mean reproduction rate was 32 individuals per day (± 3). A significant positive correlation was also found between aphid reproduction and wheat shoot N content (Table 1; Fig. 2). Using sequential decomposition of variance in the model, an effect of the presence of earthworms was shown on aphid reproduction that remained significant after considering the effect of N content (Table 1). This suggested that the

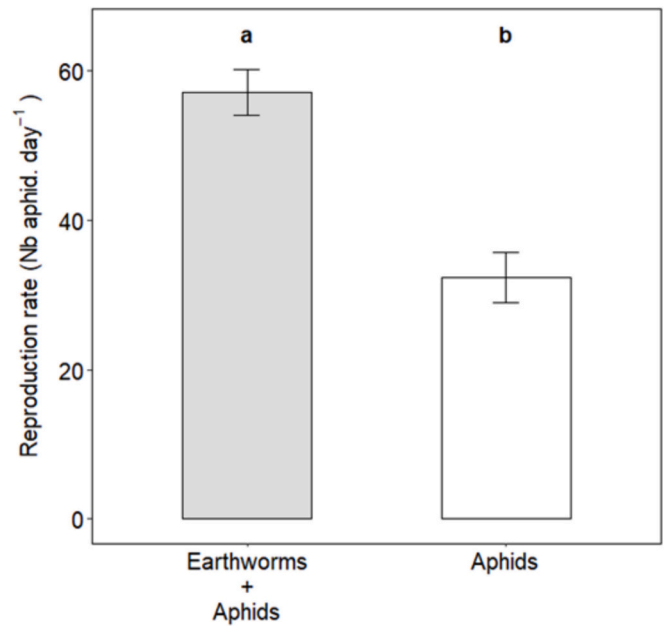


Fig. 1. Effect of the presence of earthworms on the aphid reproduction rates (Nb aphid.day⁻¹; means \pm se; $n = 15$). Letters above bars represent outputs post-hoc tests. Different letters indicate statistically significant differences between the presence (*Earthworms + Aphids*) vs the absence of earthworms (*Aphids*).

aphid reproduction rate was linked with shoot quality of wheat.

3.2. Effects of earthworms and aphids on wheat traits

A significant effect of earthworms and aphids on biomass (roots and shoots) and C and N contents in shoots was identified (Table 1; Figs. 3; 4). Regardless the presence of aphids, earthworms had a significant positive effect on root biomass (Table 1; Fig. 3). In absence of both organisms the mean (\pm se) root biomass was 0.91 ± 0.06 g; while in presence of only aphids, it was 0.85 ± 0.07 g. The mean root biomass was 1.8 times higher in presence of earthworms (1.51 ± 0.09 g with earthworms only; 1.36 ± 0.06 g with both, earthworms, and aphids). The difference of root biomass between pots with earthworms only and with both organisms was not significant, as well as the difference between pots without organisms and with only aphids.

A significant effect of both organisms on shoot biomass was also

Table 1

Summary statistics of models testing the effects of the presence of earthworms, aphids, and both (two factors with 2 treatments) on wheat traits (A) and testing the relationship between nitrogen content in shoots and aphid reproduction rates by considering the presence of earthworms as covariate (B). Linear models were used for all tests. Treatment corresponds to the different pots of the experiment: Without earthworms and aphids ($n = 15$), with earthworms ($n = 15$), with aphids ($n = 15$) or with both organisms ($n = 15$). Nitrogen content in shoots and the presence of earthworms were tested in the same model using a sequential decomposition of variance. The effect of treatment on aphid reproduction rates was tested only for pots containing aphids. Significant coefficients ($P < 0.05$) are in bold.

Response variable	Predictors					
	Presence of earthworms		Presence of aphids		Presence of earthworms * presence of aphids	
	F value (df)	p-Value	F value (df)	p-Value	F value (df)	p-Value
Dry root biomass (g)	63.172 (1)	<0.001	2.52 (1)	0.118	0.421 (1)	0.519
Dry shoot biomass (g)	68.76 (1)	<0.001	38.11 (1)	<0.001	5.85 (1)	0.019
C content in shoots (%)	0.00 (1)	0.949	13.19 (1)	<0.001	1.56 (1)	0.217
N content in shoots (%)	30.75 (1)	<0.001	39.94 (1)	<0.001	6.69 (1)	0.012
h						
Response variable	Predictors				Presence of earthworms	
	Shoot N content				F value (df)	p-Value
	F value (df)	p-Value			F value (df)	p-Value
Aphid reproduction rates (Nb aphid.day ⁻¹)	26.90 (1)	<0.001			8.34 (1)	0.008

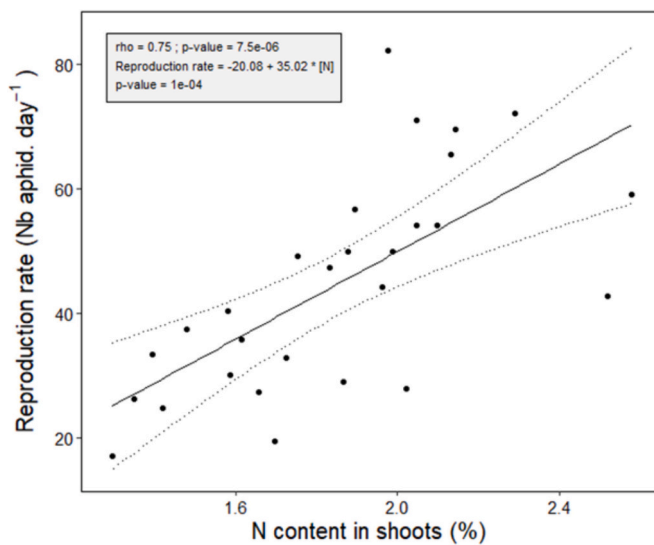


Fig. 2. Correlation between aphid reproduction rates (Nb aphid.day⁻¹) and N content in shoots. Dots represent each pot with aphids. Regression line and dotted lines represent predictions from linear models and corresponding confidence interval. Spearman's correlation coefficients, rho, and the equation of regression line and their *p*-values are indicated.

observed (Table 1; Fig. 3). The presence of earthworms had a significant positive effect on shoot biomass (3.20 ± 0.12 g) (Table 1; Fig. 3). It was 1.4, 1.7 and 1.3 times higher than wheat shoot biomass without organism (2.19 ± 0.09 g), with aphids only (1.83 ± 0.08 g) and with both organisms (2.39 ± 0.08 g), respectively (Fig. 3). The difference in wheat shoot biomass between pots without organisms and pots with both organisms was not significant (Fig. 3). The shoot biomass for pots with only aphids was significantly lower than the other treatments (Fig. 3).

Regarding C and N contents in shoots, a significant difference was observed between treatments (Table 1, Fig. 4). Even though the C content was very similar between the different treatments, plants grown in the presence of only aphids (40.52 ± 0.29 %) had a significantly lower carbon content than the others (except for the pots containing both organisms, 40.89 ± 0.37 %) (Fig. 4). Nitrogen content was significantly higher in

plants grown in the presence of earthworms and aphids (2.07 ± 0.07 %) than in other treatments (Fig. 4), corresponding to an increase of 43 % compared with the control without organisms. Conversely, the N content in plants grown without organisms was significantly lower (1.45 ± 0.03 %) than in the other treatments (Fig. 4). There was no significant difference between plants grown on pots containing only earthworms (1.58 ± 0.04 %) and pots containing only aphids (1.63 ± 0.07 %) (Fig. 4). This corresponded to an increase of 9 % and 12 % respectively compared with the control without organism.

4. Discussion

4.1. Effects of earthworms and aphids on wheat traits

This study brings new insights on the interactions between the below and aboveground compartments. The results showed significant effects of below and aboveground organisms on plant traits. Indeed, wheat biomass and carbon and nitrogen contents were influenced by the presence of earthworms and aphids. Regardless of the presence of aphids, the results showed a higher root biomass in the presence of

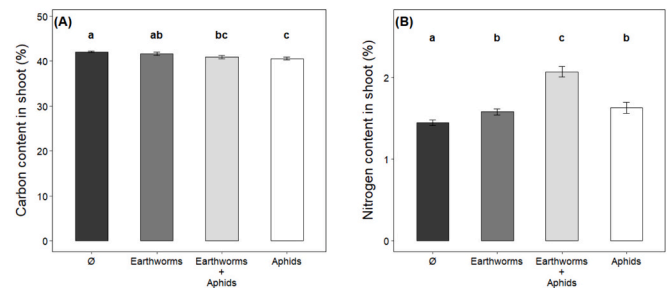


Fig. 4. Effect of the presence of earthworms ($n = 15$), aphids ($n = 15$), and both ($n = 15$) on carbon (A) and nitrogen (B) contents in shoots. Bar and vertical error bars represent raw means \pm se. Letters above bars represent outputs of post-hoc tests. Different letters indicate statistically significant differences between the treatments: Ø = no earthworms and no aphids; Earthworms = presence of earthworms and no aphids; Earthworms + Aphids = presence of earthworms and aphids; Aphids = no earthworms and presence of aphids.

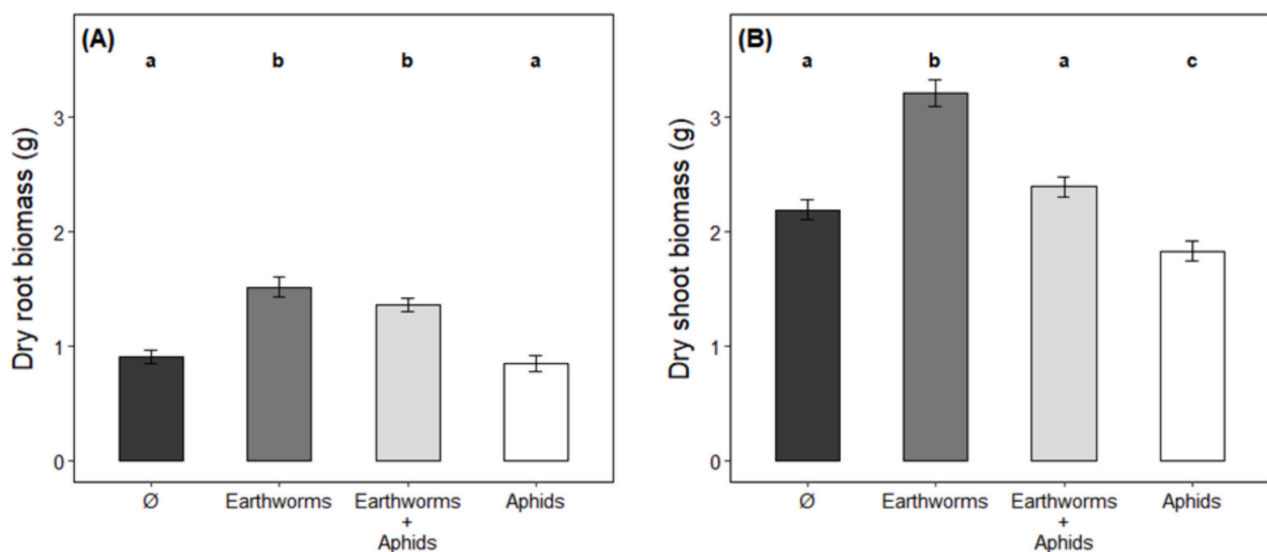


Fig. 3. Effect of the presence of earthworms ($n = 15$), aphids ($n = 15$), and both ($n = 15$) on wheat traits: dry root (A) and shoot (B) biomass. Bar and vertical error bars represent raw means \pm se. Letters above bars represent outputs post-hoc tests. Different letters indicate statistically significant differences between the treatments: Ø = no earthworms and no aphids; Earthworms = presence of earthworms and no aphids; Earthworms + Aphids = presence of earthworms and aphids; Aphids = no earthworms and presence of aphids.

earthworms, suggesting that, unlike aphids, earthworms stimulate root growth. This could be explained, for instance by the activity of earthworms, which increase soil porosity, leaving more space for root growth and prospection (Bertrand et al., 2015; Brown et al., 2004; Edwards and Lofly, 1980) as well as in improving nutrient availability, resulting in a better acquisition of resources by roots (Xiao et al., 2018) or a beneficial change for the plant in the communities of micro-organisms (Blouin et al., 2005; Eisenhauer, 2010; Hodson et al., 2023; Medina-Sauza et al., 2019). In addition, earthworms had also a positive effect on wheat shoot biomass, suggesting that earthworms can promote both aboveground and belowground plant growth likely by furthering resource acquisition (Xiao et al., 2018). By contrast, aphids alone had no effect on root biomass and even reduced aboveground plant growth by eating phloem sap and interfering with the photosynthesis process. Interestingly, in the presence of earthworms, the detrimental effect of aphids on shoot biomass was lowered as plants with both organisms showed similar shoot biomass than plants without organisms. This suggests that the negative effect of aphids on plant growth could be counterbalanced by the positive effect of earthworms. These results highlight the importance of soil fauna in controlling the negative effects of pests in plant growth (Bezemer and Vandam, 2005; Scheu, 2003; Wurst, 2010).

Both organisms had a significant effect on the uptake of C and N by shoots. Carbon content was lower in the presence of aphids. Aphids likely reduced carbon acquisition by interfering with the metabolic process (Giordanengo et al., 2010; Guerrieri and Digilio, 2008; Nietupski et al., 2022). By contrast positive effect of earthworms on C acquisition might result from their stimulating effect on plant growth which in turn requires higher photosynthetic rate, allowing to compensate for the negative effect of aphids. Interestingly, the nitrogen content was significantly higher in the presence of aphids or earthworms alone (with no difference between these two treatments) and that it was even higher when the two organisms were present together. Higher root biomass, as well as higher N mineralization, in the presence of earthworms might explain higher acquisition of nitrogen from the soil by plants (Andriuzzi et al., 2016; Haase et al., 2008). In the presence of aphids, earthworms likely allowed better nitrogen acquisition by plants to produce nitrogen-based defences against aphids which explains higher N concentration in plants grown in the presence of both aphids and earthworms. These results highlight the importance of soil fauna in the plant's response to aboveground pests. Indeed, in response to pest insect attacks, and in the presence of soil fauna, the plant could induce a greater production of chemical defences (Bezemer and Vandam, 2005; Frost et al., 2008; Pineda et al., 2010; Van Wees et al., 2008; Wurst, 2010). Interestingly, the positive role of earthworms on plant growth and defence induction could be indirect through changes in the community of microorganisms (Blouin et al., 2005; Eisenhauer, 2010; Hodson et al., 2023; Medina-Sauza et al., 2019). For example, Hodson et al. (2023) showed that the effect of earthworms on plant growth was due to changes in the microbiome that could also affect the production of defences against pests.

A wide variety of secondary defence compounds are known to exist in plants (Dixon and Strack, 2003; Wink, 2008). Resource allocation to defences could be a response to insect attacks (Frost et al., 2008). For example, the production of insecticidal peptides or proteins is one of the main direct defence reactions of plants against attack by herbivorous insects (Carlini and Grossi-de-Sá, 2002; Nalam et al., 2019; Vandenborre et al., 2011). Thus, several nitrogen-containing defences could explain this increase in nitrogen content. Phloem sap also contains lectins, which bind specifically to carbohydrates in the insect guts and interfere with physiological processes, affecting the insect health (Carlini and Grossi-de-Sá, 2002; Vandenborre et al., 2011; Vasconcelos and Oliveira, 2004). Therefore, for further study, it would be interesting to refine the chemical analyses, in order to differentiate the nitrogen compounds and see whether or not the increase in wheat N content is due to the induction of defence compounds. An analysis of the microbial community would also provide a better understanding of the effects of earthworms

on wheat traits and its response to pest attacks.

4.2. Effects of earthworms and wheat nitrogen content on aphid reproduction

However, the results showed a strong effect of earthworms on aphids, with a reproduction rate almost twice as high as in the absence of earthworms, which is consistent with the literature (Eisenhauer and Scheu, 2008; Wurst and Jones, 2003). This could be explained by the amount of available biomass for the insects. Indeed, earthworms have a positive effect on aboveground biomass, which could consequently reduce competition for aphids and thus, increase reproduction rates (Ke and Scheu, 2008; Poveda et al., 2005). However, no winged aphids were counted in any treatment, suggesting that there was little or no stress during the experiment (Guerrieri and Digilio, 2008; Müller et al., 2001). Furthermore, a positive correlation was found between aphid reproduction rate and plant nitrogen content, confirming observations in the literature showing a positive relationship between plant nitrogen content and insect herbivore performance (Aqueel and Leather, 2011; Haase et al., 2008; Schütz et al., 2008; Wurst and Jones, 2003). Thus, in addition to be driven by the production of N-containing defences, N content in plants might also be controlled by the needs of aphids for their reproduction.

Aphids receive all their nutrients from phloem sap that contains high concentrations of sugars, and nitrogen is available mainly in the form of free amino acids (Douglas, 2006). Despite this, the quantity of essential amino acids seems insufficient to the aphid needs, which is compensated for by a bacterial endosymbiotic which provide some amino acids essential in animal diets from the phloem sap (Douglas, 2006; Hansen et al., 2020). However, in addition to these adaptations, it has also been shown that aphids are able to modify the host plant metabolism in order to adapt the composition of phloem sap to their nutritional needs, and thus, modify nitrogen allocation (Giordanengo et al., 2010; Gironse et al., 2005; Züst and Agrawal, 2016). This ability could explain the higher nitrogen content in the presence of aphids, and their higher reproduction rates, enhanced by the presence of earthworms. Although the aphid reproduction rate is higher in the presence of earthworms, the essential function of earthworms in biomass production has been highlighted, which remains more important than in their absence, even though fewer aphids are observed under this treatment. The negative effect that such a high number of aphids could have on wheat biomass is therefore compensated for by the activity of earthworms. Furthermore, as mentioned by Xiao et al. (2018), in their meta-analysis the effects of earthworms on plant resistance against herbivores vary considerably between studies. For example, Ke and Scheu (2008) showed a negative effect of endogeic earthworms *Aporrectodea caliginosa* on aphid *Rhopalosiphum padi* reproduction, while Eisenhauer and Scheu (2008) showed a positive effect of the anecic earthworm *Lumbricus terrestris* on the same aphid species, as we have shown in the present study with *Sitobion avenae*. Therefore, for further study, it would be interesting to investigate the effect of the combination of the two ecological types, which are mainly present in crop fields (endogeic and anecic), which should result in better resource acquisition via niche complementarity.

Moreover, although nitrogen content and aphid reproduction were significantly correlated, the effect of earthworms on aphid performance remained significant after nitrogen content was accounted for as a covariate in statistical models. This result indicates that differences in herbivore performance between the presence and absence of earthworms depend on other factors than nitrogen content solely. For instance, the presence of earthworms could affect chemical or physical defensive traits which could drive the herbivore response (Blouin et al., 2013, 2005; Hodson et al., 2023; Wurst, 2010). Therefore, leaf tissue N content is not necessarily the most appropriate trait to detect nitrogen availability for aphids and to test the effects of soil organisms on aphid performance. Further studies on the chemical composition of the phloem would provide additional insights into the interaction between

earthworms, plants, and aphids. Also, a simplified calculation of the reproduction rate was used by dividing the final number of aphids by the total number of days in the experiment. Therefore, for further study, it would be preferable to count the number of aphids each day to obtain a more realistic reproduction rate and introduce a mortality rate.

5. Conclusion

Overall, this study showed that earthworms have important effects on plants and indirectly on aphids. These results have implications for crop management, as they indicate that the presence of earthworms may contribute to reduce the detrimental effects of aphids on plant biomass, probably by acting on nutrient uptake and plant defensive traits. These indirect trait-mediated effects highlight the importance to study pest management with a more holistic view of agroecosystems. This study brings new understanding of the interaction between soil, plant and herbivores and shows that the belowground compartment cannot be treated as a simple black box whose role is limited to replacing nutrients for plant growth. Soil organisms affect herbivore performance by changing resource uptake or inducing plant defences. The lack of integration of studies on effects of soil organisms on plant performance and on the whole aboveground system opens a large scope for future research for crop protection. It is imperative to have an ecological perspective in studying the relationships between soil organisms and plants and to consider soil organisms as driving factors of the aboveground food web. In conclusion, by addressing the effect of earthworms on both the pest performances and plant traits, the need for further research into the indirect effects of belowground compartments on pest performance is highlighted.

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CRediT authorship contribution statement

Thomas Damestoy: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **David Houben:** Writing – review & editing, Validation, Supervision, Formal analysis. **Léa Kervroëdan:** Writing – review & editing, Validation, Supervision, Project administration. **Laurine Ambeza:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Emma Pluchard:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Anne-Maïmiti Dulaurent:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

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.

Data availability

A preprint version of the dataset has already been published on Dryad Digital Repository and can be accessed at: doi:<https://doi.org/10.5061/dryad.fttdz0912>.

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