Plants interact with a large diversity of microbes and insects, both below and above ground. While studies have shown that belowground microbes affect the performance of plants and aboveground organisms, we lack insights into how belowground microbial communities may shape interactions between aboveground pathogens and insects. We investigated how soil microbiomes and aboveground organisms affect plant growth and development, and whether differences in soil microbiomes influence interactions between aboveground organisms. We conducted a growth-chamber experiment with oak seedlings *Quercus robur* growing in three soils with similar abiotic soil properties but with distinct natural soil microbiomes. Seedlings were subjected to single or dual attack by powdery mildew *Erysiphe alphitoides* and aphids *Tuberculatus annulatus*, either in the presence or absence of prior attack by a free-feeding caterpillar *Phalera bucephala*. Soil microbiomes were associated with differences in seedling height, and seedlings with multiple aboveground organisms had more but smaller leaves than healthy seedlings. The soil microbiome affected the severity of powdery mildew infection, and mediated the impact of co-occurring aboveground organisms on aphid population size. Our study highlights that plant performance is affected by natural soil microbiomes as well as aboveground organisms, and that natural soil microbiomes can affect interactions between pathogens and insects. These findings are important to understand species interactions in natural systems, as well as for practical applications, such as manipulation of soil microbiomes to manage agricultural pests and diseases.

Keywords: aboveground–belowground interactions, herbivorous insects, pedunculate oak, plant–microbe–insect interactions, soil microbiomes

---

**Introduction**

Plants, by being rooted in soil and growing in air, form a connection between the below- and aboveground world. In both worlds, plants are interacting with a large diversity of arthropods and microbes (De Deyn and Van der Putten 2005). Several studies have shown that aboveground interactions between pathogens and insects are important for plant performance (Hauser et al. 2013), attacker performance
The outcome of these aboveground plant–pathogen–insect interactions can be highly context dependent (Tack and Dicke 2013). For example, abiotic conditions, like air temperature and soil moisture, are known to have a strong effect on the outcome of aboveground interactions via impacts on plant defences (Bultman and Bell 2003, Pineda et al. 2013) or the plant phyllosphere (Gong and Xin 2021). We also know that the belowground microbial community plays an important role in plant performance and resistance to aboveground herbivores and pathogens (Bennett and Bever 2007, Pineda et al. 2010). However, few studies have looked into how soil microbiomes can influence aboveground plant–pathogen–insect interactions, and this is especially true for natural systems (Hartley and Gange 2009, Castagneyrol et al. 2018). Such knowledge is important for our understanding of spatial and temporal variation in the outcome of plant–pathogen–insect interactions in nature, and can inform strategies that use soil microbiomes to sustainably manage agricultural pests and diseases.

Soil microbial communities may directly affect plant performance (Smith and Read 2010). For example, mycorrhizal fungi and nitrogen-fixing bacteria can enhance nutrient uptake by plant roots and thereby promote plant growth (Smith and Read 2010, Hart and Forsythe 2012), whereas soil-borne pathogens may hamper plant performance (Berendsen et al. 2012). Soil microbes can also indirectly affect plant performance by altering a plant’s tolerance or resistance to antagonistic organisms (Pineda et al. 2010, Zamioudis and Pieterse 2011). For example, the increase in nutrient uptake through an association with beneficial soil microbes may increase the plant’s ability for compensatory growth (Bennett and Bever 2007). On the other hand, more nutritious plant tissues may also increase insect pest and disease levels and thereby impair plant performance (Hoffland et al. 2000, Hoffmann et al. 2009). Beneficial micro-organisms, such as growth-promoting rhizobacteria, can sensitize the immune system of their host plant by inducing systemic resistance (Wilkinson et al. 2019), thus rendering the plant more resistant to attackers (Rashid and Chung 2017). The induction of these defence pathways is dependent on the type of antagonist: The SA pathway targets biotrophic pathogens and sap-sucking insects, whereas the JA pathway is effective against necrotrophic pathogens and chewing insects (Walling 2000, Thaler et al. 2012). As these defence pathways are known to influence one another via phytohormonal cross-talk (Pieterse et al. 2012), soil microbes may strongly influence the outcome of interactions among aboveground organisms (Castagneyrol et al. 2018). Yet, no study to date has examined the impact of natural soil microbiomes and aboveground pathogens and insects were associated with differences in plant performance, and whether soil microbiomes could differentially influence interactions between aboveground pathogens and insects via induced plant responses. To this end, we conducted a multifactorial climate chamber experiment in which we grew pedunculate oak seedlings Quercus robur with three distinct natural soil microbiomes, and exposed the seedlings to either none, one, two or three aboveground organisms. The aboveground organisms included were a biotrophic pathogen (powdery mildew, Erysiphe alphioides), aphids Tuberculatus annulatus and chewing caterpillars Phalaena bucephala. Our main objective was to explore how distinct natural soil microbiomes influence aboveground plant–pathogen–insect interactions, rather than to identify the functional role of specific microbial taxa. Specifically, we asked the following questions.

1) Do natural soil microbiomes differ in their effects on host plant performance? And do aboveground insects and pathogens affect plant performance?
2) Do natural soil microbiomes differ in their effects on interactions between aboveground pathogens and insects?
Based on that soil microbial communities have been shown to affect the tolerance and resistance of plants to aboveground organisms (Pineda et al. 2010, Zamiodus and Pieterse 2011, Kurth et al. 2014), we hypothesized that the effects of below- and aboveground organisms on plant performance will be interactive. More specifically, the effect of aboveground organisms on seedling performance will depend on the soil microbiome a seedling is growing with, e.g., due to differences in the seedling's resource budget or induced defences. We further expected that differences in soil microbiomes lead to different outcomes of interactions between aboveground pathogens and insects.

**Material and methods**

**Study system**

The pedunculate oak *Quercus robur* (Fagaceae) is a broad-leaved deciduous tree that is occurring in large parts of Europe, with its northern range edge in central Sweden (Repo et al. 2008). Oaks harbour a large diversity of insect herbivores and microbes, and the insects include both chewing caterpillars and suckling insects (Southwood et al. 2004). Chewing caterpillars are typically the ones arriving early in the growing season (i.e. early spring in temperate climates) (Feeny 1966, Forkner et al. 2004). Among the chewing insects is the buff tip moth *Phalera bucephala* (Notodontidae). Female moths oviposit their egg clutches in early summer (June–July), and the hatching caterpillars feed on several deciduous tree species, including oak (Waldbauer 1968). The larval stage can last for up to two months. The caterpillars pupate in the soil where they remain during winter, and eclose the following spring as adult moths (Patočka and Tůrčán 2005). Another insect herbivore on oak is the common oak aphid *Tuberculatus annulatus* (Aphididae), which is a sucking insect that feeds on the phloem sap of oak leaves. During the growing season, aphids reproduce asexually and population sizes can increase rapidly when conditions are favourable (Moran 2002). By the end of the growing season, females reproduce sexually and oviposit eggs that hibernate till the next growing season. Besides herbivores, oaks are also attacked by several pathogens, of which the most common one is the oak powdery mildew, *Erysiphe alphitoides* (Desprez-Loustau et al. 2018). This species grows epiphytically, with only its haustoria penetrating epidermal cells to extract nutrients. During the growing season, the pathogen has multiple generations with wind-dispersed, asexual spores. At the end of the growing season sexual spores are formed, which overwinter in tree bark, buds or leaf litter until the next growing season (Marçais et al. 2009).

**Collection and preparation of soils, plants, pathogen and insects**

To include a broad range of variation in natural soil microbiomes in our experiment, we collected natural soils of different types from three separate geographic locations (site A, B and C, which were ~1.0–1.5 km apart) in nature reserve Norra Djurgården, Stockholm. We ensured that oaks were naturally occurring at each site. Site A was a deciduous forest where soil was mostly sandy (59°21’52.5"N, 18°02’57.0"E), site B was a mixed deciduous forest where the soil was a mix of clay and sand (59°22’07.6"N, 18°04’15.1"E), and site C was a grass meadow, where the soil contained mostly clay (59°22’07.7"N, 18°02’59.8"E). We then mixed equal amounts of soil from the three different sites (1:1:1), whereby the soil from two of three sites was sterilized by autoclaving (soils were not sieved) (Fig. 1a). In this way, we created 3 soil mixes, each with 1 part unsterilized and 2 parts sterilized soil (referred to as soil mixes A, B and C, respectively, according to the origin of the non-sterilized soil). Soil mixes thus differed in their biotic, but not abiotic, characteristics, which ensured that any soil effects observed in this experiment were caused by differences in the biotic compositions rather than in soil texture or fertility. Since we aimed to study the aboveground consequences of differences in soil microbiomes, and not the effect of the presence of a soil microbiome per se, we did not include a treatment consisting of a mix with only sterile soils (Bezemer et al. 2006, Kardol et al. 2006, Tack et al. 2015). We filled 7 × 7 × 18 cm, 700 ml pots with 300 ml sterilized and nutrient-poor potting soil, 125 ml of natural soil mix (soil mix A, B or C), again 250 ml sterilized potting soil, and topped off with 25 ml sterilized sand (Fig. 1a) (method adopted from Tack et al. 2015 and Rasmussen et al. 2020, modified to our study system). In total 300 pots were filled, that is, 100 pots per soil microbiome. Henceforth, soil microbiomes will be referred to as soil A, B and C, respectively. To characterize the microbial composition of each of the three soils, we used amplicon sequencing of the ITS and 16S regions to identify fungal and bacterial taxa, respectively. Three weeks after pots were filled with soil A, B or C, we took soil cores (1 cm in diameter, to a depth of 10 cm) of 15 randomly selected pots for each soil microbiome. Each soil sample (i.e. an individual soil core) was thoroughly mixed, after which we stored 2 ml of each sample in a sterile Eppendorf tube at −20°C. For details on sequencing methods and bioinformatic see the Supporting information. After fungi were identified, we assigned functional groups based on the FungalTraits database (Põlme et al. 2020).

Acorns (nuts that contain oak seeds) were collected from Stockholm area in the autumn of 2018 and stored in a cold room during winter before being planted. Once a seedling had developed its first leaves (approximately 1 week after leaves appeared, mostly 3–5 leaves per seedling), it was included in the experiment. Aphids *Tuberculatus annulatus* were collected from natural populations in Stockholm in spring 2018, and were reared on oak saplings in a climate chamber (10 h 20°C: 14 h 18°C, light: dark) for several generations prior to the experiment. Powdery mildew *Erysiphe alphitoides* originated from naturally occurring powdery mildew colonies in Stockholm, and was maintained in the greenhouse on oak saplings (10 h 20°C: 14 h 18°C, light: dark). Caterpillars of the buff-tip moth *Phalera bucephala* were ordered from
Worldwide Butterflies (UK), and were fed for several days on oak leaves before being used in the experiment.

**Experimental design and introduction of aboveground organisms**

To investigate the effect of soil microbiomes on interactions between seedlings and aboveground organisms, as well as interactions among aboveground organisms, we randomly assigned oak seedlings of the three soil microbiomes to 5 different combinations of aboveground organisms (henceforth referred to as ‘treatments’): 1) healthy oak seedlings, 2) only mildew, 3) only aphids, 4) mildew and aphids simultaneously and 5) first a caterpillar, then mildew and aphids simultaneously (Fig. 1c). We included 20 oak seedlings in each combination of soil microbiome and treatment, resulting in 100 plants per soil microbiome and a total of 300 seedlings. During the experiment, all seedlings were kept in a climate chamber (10 h 20°C: 14 h 18°C, light: dark, light intensity 110 µmol m⁻² s⁻¹, air humidity 65%) and covered by a pollination bag (duraweb material, PBS international, Scarborough, UK) to prevent the spread of aboveground organisms among seedlings. The experiment lasted for six weeks.

Oak seedling height, number of shoots, number of developed leaves and size of the largest leaf (leaf length × leaf width, henceforth referred to as ‘leaf size’) were recorded at the start and end of the experiment (cf. van Dijk et al. 2020). We further estimated total leaf area according to the following formula:
Total leaf area

\[ \text{Total leaf area} = \sqrt{\text{size of the largest leaf} \times \text{number of developed leaves}} \]

Henceforth, these different estimates of plant growth and development will collectively be referred to as ‘plant performance’. Non-destructive measurements of oak seedling traits, such as the ones used in our study, have been found to correlate strongly with seedling biomass, though investments in different plant parts might depend on the environmental conditions (Gardiner and Hodges 1998).

Oak seedlings were infested with one caterpillar (2nd instar) at the start of the experiment (week 1) (Fig. 1c). The caterpillars were allowed to feed on the seedling for one week to cause a significant amount of feeding damage, after which they were removed. Oak seedlings were infested with five wingless aphid nymphs in week 3 of the experiment (Fig. 1c). In case an aphid population was observed to drop below this number, additional aphids were introduced to maintain a minimum of five aphids per seedling (van Dijk et al. 2020). As a measure of aphid performance, the total number of aphids on a seedling was counted every week. Oak seedlings were infected with mildew in week 3 by gently brushing all leaves with mildew spores originating from the greenhouse maintained mildew colonies (Fig. 1c) (Mursinoff and Tack 2017, van Dijk et al. 2020). Mildew inoculation was repeated until symptoms of disease were observed, with infection being visible as white colonies (mycelium and spores) on the upper side of the leaves. As a measure of mildew performance, the total percentage of mildew cover was estimated for all infected seedlings every week by the same person.

Statistical analyses

Statistical analyses were performed in R ver. 3.6.3 (<www.r-project.org>). We used the package lme4 to run linear mixed models (Bates et al. 2015), the Anova function in the car package to assess significance of the models (Fox and Weisberg 2019), and emmeans to estimate specific contrasts in our models (with Tukey adjustment) (Lenth 2020). For multivariate models, we used the adonis2 function in the vegan package (Oksanen et al. 2020).

Soil microbial richness and community composition

To examine whether soils differed in their fungal and bacterial richness (i.e. number of ASVs), we modelled fungal and bacterial richness as a function of soil type. To investigate differences in microbial community composition between the soil types, we modelled multivariate fungal and bacterial community composition as a function of soil type, using normalized count data (CSS) with Bray–Curtis dissimilarity metrics. To investigate which taxa differed in relative abundance (i.e. relative number of reads per taxon) between soils, we conducted a differential abundance analysis using a non-parametric Kruskal–Wallis test (Kruskal and Wallis 1952). p-values were adjusted with the Bonferroni correction to account for multiple comparisons.

Effects of soil microbiomes and aboveground organisms on plant performance

To examine whether distinct soil microbiomes and aboveground organisms were associated to plant performance, we modelled plant traits (plant height, number of shoots, number of developed leaves, size of largest leaf and total leaf area) as functions of soil microbiome, treatment and their interaction. To account for maternal effects, we included the value of the focal plant trait at the start of the experiment as a covariate in all plant performance models (for details on all the plant models see the Supporting information).

Effects of soil microbiomes on interactions between aboveground organisms

To examine the effect of soil microbiomes and co-occurring aboveground organisms on mildew infection severity and aphid population growth, we modelled the percentage of seedling covered by mildew mycelium and the total number of aphids on a seedling as functions of soil microbiome, treatment, date and their interactions. To account for repeated measures of the aboveground organisms on the same set of seedlings, we included seedling ID as a random effect in the model (Supporting information). If there was a significant interaction between date and soil or treatment, we ran date-specific models to identify the effects of soil and treatment on aphid and mildew performance during specific weeks (Supporting information). Likewise, if there was a significant interaction between treatment and soil, we ran soil-specific models to identify the effect of treatment separately for each soil community (Supporting information).

Results

Soil microbial richness and community composition

Soil microbiomes significantly differed in fungal richness ($F_{2,42} = 5.25, p = 0.01$): soil C had significantly higher richness than soil B ($t_{42} = -3.15, p = 0.01$), while fungal richness in soil A was in between that of soil B and C (Supporting information). The fungal composition also differed among the three soil types (Supporting information), with soil type explaining 27% of the variation in fungal communities ($p = 0.001$). Regarding fungal communities, soil C was dominated by Ascomycota and soil B by Basidiomycota, while these phyla had similar relative abundances in soil A (Supporting information). Of the identified fungal genera, Coniochaeta and Pseudorototium were dominant in soil C, while these genera had much lower relative abundances in soil A and B (Supporting information). Taken together, the soils had significantly different relative abundances for 29 fungal taxa ($p < 0.05$, Supporting information). Most of the characterized fungal taxa were putative saprotrophs, but we also detected mycorrhizas (soil B), plant pathogens (soil A and C), ectomycorrhizal fungi (soil B) and root endophytes (soil A) (Supporting information).

Soil microbiomes did not significantly differ in their bacterial richness ($F_{2,42} = 1.81, p = 0.18$) (Supporting information). However, the bacterial composition differed among the three
soil types (Supporting information), with soil type explaining 31% of the variation in bacterial communities (p = 0.001). The relative abundances of bacterial phyla were rather similar among soils (Supporting information), though there were slight differences in the relative abundance of bacterial genera (Supporting information). The soils had significantly different relative abundances for 89 bacterial taxa (p < 0.05; taxa with p < 0.005 are shown in the Supporting information).

Effects of soil microbiomes and aboveground organisms on plant performance

Oak seedling performance was affected by soil microbiome identity and aboveground organisms, but not their interaction (Fig. 2, Supporting information). Soil microbiome identity was associated to seedling height, where seedlings growing with soil microbiome A were taller than seedlings growing with soil microbiome C (t = 2.60, p = 0.03; Fig. 2a, Supporting information). Soil microbiome identity was not associated with the number of shoots, leaf number, leaf size or leaf area (Fig. 2e–j). Seedlings attacked by three aboveground organisms had more shoots than healthy seedlings (treatment 1 versus 5, t = −3.00, p = 0.02, Fig. 2d, Supporting information), but tended to be shorter (Fig. 2b, Supporting information). Seedlings attacked by multiple aboveground organisms tended to have smaller, but more, leaves (treatment 1 versus 4, t = 2.77, p = 0.05, and treatment 1 versus 5, t = −2.53, p = 0.09, respectively, Fig. 2f–h, Supporting information). The responses of these traits were in opposite direction, and there was no effect of aboveground organisms on total leaf area (Fig. 2, Supporting information).

Effects of soil microbiomes on interactions between aboveground organisms

Soil microbiome identity affected mildew performance (Fig. 3a, Supporting information), with seedlings growing in soil C experiencing higher mildew cover than seedlings in soil B (t = −2.50, p = 0.04). Aboveground organisms and the interaction between aboveground organisms and soil microbiome did not influence mildew performance (Fig. 3b, Supporting information).

Aphid performance was not affected by the independent effects of soil microbiome or co-occurring aboveground organisms, but only by their interaction, or, in other words, the effect of co-occurring organisms on aphids was dependent on the soil microbiome (Fig. 4, Supporting information). When the host plant was growing with soil microbiome A, aphids performed best when they were the only aboveground organism on the host plant, whereas aphid performance was significantly reduced when sharing a caterpillar-damaged plant with mildew (t = 2.85, p = 0.02, Fig. 4c). Aphids that attacked seedlings growing in the other two soils were not significantly affected by co-occurring aboveground organisms (Fig. 4c). The direction of these effects remained the same over the experiment, even though there was some variation in the strength of the effect between weeks (Supporting information).

Discussion

Our study showed that distinct soil microbiomes are associated with differences in aboveground plant–pathogen–insect interactions in natural systems. Plant performance was affected by the soil microbiome and aboveground organisms, but not their interaction. The severity of pathogen infection was dependent on the composition of the soil microbiome, whereas aphid population size was influenced by the interactive effect of soil microbiome and co-occurring aboveground organisms. Taken together, our results show that soil microbiomes affect the performance of plants and aboveground organisms, and, interestingly, also shape the interactions between aboveground organisms. These findings highlight the role of soil microbiomes in shaping aboveground plant–pathogen–insect interactions, which increases our fundamental understanding of plant food webs and may inform management strategies that aim to sustainably control agricultural pests and diseases via the manipulation of soil microbial communities.

Effects of soil microbiomes and aboveground organisms on plant performance

Soil microbiome identity was associated to some aspects of plant performance. Height of the seedlings differed among soil microbiomes, where seedlings growing in soil A were taller than seedlings growing in soil C. The impact of natural soil microbiomes on plant performance can vary considerably (Bennett and Bever 2007, St-Denis et al. 2017). Previous studies showed that the impact of natural soil microbiomes on plant performance can depend on species diversity (van der Heijden et al. 1998, Bonkowski and Roy 2005) and composition (Jonsson et al. 2001, Williams et al. 2014) of the soil microbial community. Commonly, the impact of soil microbes on plant performance is studied using single microbial strains (Garcia et al. 2004), species (Dehlin et al. 2008) or taxa (Egerton-Warburton and Allen 2001). While this approach allows for pinpointing the mechanistic roles of particular soil microbes, it might not provide us with insights into how natural soil microbial communities can influence plant performance. Studies of plant–soil feedbacks have made it increasingly clear that effects of soil communities on plant performance are difficult to deduce from the additive effects of specific microbial taxa (Larimer et al. 2010, Berendsen et al. 2012). Thus, studies with a focus on natural soil communities are important to better understand the diverse effects that natural soil communities can have on plants. One interesting avenue for future research is to study the influence of soil microbial legacies by conducting experiments with natural soils collected from plant communities with different characteristics, e.g. low versus high densities of the focal plant.

We found that plants with multiple aboveground organisms had smaller leaves, but also tended to have more leaves. The responses of these traits were thus in opposite directions, resulting in the absence of an overall effect on total leaf
Figure 2. The effect of soil microbiomes and aboveground organisms on plant performance. The panels show the effect of soil microbiome (left) and aboveground organisms (right) on plant height, number of shoots, number of developed leaves, leaf size (length × width of the largest leaf) and total leaf surface, respectively. Soil microbiomes include soil A, B or C, and aboveground-organism treatments include: 1) healthy oak seedlings, 2) seedlings attacked by mildew, 3) seedlings attacked by aphids, 4) seedlings attacked by mildew and aphids and 5) seedlings first attacked by caterpillars, then by mildew and aphids. Shown are least squares means ± standard errors, and letters indicate which bars significantly differ from each other (p < 0.05). Note that the y-axis does not start at zero in (a) and (b). For a visualization of (non-significant) interactive effects of soil microbiome and aboveground organisms on plant performance see the Supporting information. For statistics related to this figure, including test statistics, degrees of freedom and p-values and information on model structures, see the Supporting information.
Figure 3. The effect of soil microbiome and co-occurring aboveground organisms on mildew infection severity, i.e. the percentage of mildew coverage on a seedling’s leaves. (a) shows the effect of soil community on mildew infection severity over time (from week 4 to 7). (b) shows the effect of treatments with aboveground organisms on mildew infection severity over time (from week 4 to 7). Error bars represent standard errors. Significant differences (p < 0.05) between treatments in each week are indicated by letters above the bars. For statistics related to this figure, including test statistics, degrees of freedom and p-values and information on model structures, see the Supporting information.

Figure 4. The effect of soil microbiome and co-occurring aboveground organisms on aphid population size. (a) shows the effect of soil microbiome on aphid population size over time (from week 4 to 7). (b) shows the effect of aboveground organisms on aphid population size over time (from week 4 to 7). (c) shows the combined effect of soil microbiome and treatment on aphid population size in week 7. Error bars represent standard errors. Significant differences (p < 0.05) between treatments in each week are indicated by letters above the bars. For statistics related to this figure, including test statistics, degrees of freedom and p-values and information on model structures, see the Supporting information.
area. This result corresponds to the findings of Hilton et al. (1987), who detected more but smaller leaves on oak seedlings Q. robur that experienced severe defoliation. We also found that plant height and number of shoots tended to be affected in opposite directions, as plants were smaller but had more shoots in the presence of multiple aboveground organisms. These findings suggest that plants change their growth pattern in response to the presence of multiple aboveground organisms. Woody plants have been reported to shift the allocation of biomass when experiencing resource limitation or damage by aboveground organisms (Stevens et al. 2008, Eyles et al. 2009). In some cases, growth increases due to increased allocation of resources to new shoots (Kurth et al. 2014). On the other hand, Q. petraea and Q. robur for higher resistance against powdery mildew Erysiphe alphitoides (Kurth et al. 2014). On the other hand, soil microbes can promote powdery mildew performance by e.g. increasing the nutritional value of the host plant (Germns et al. 2001), and powdery mildew has been shown to perform better on plants growing in soils with arbuscular mycorrhizal fungi than on plants growing in control soils (Pozo and Azcón-Aguilar 2007). Effects of soil microbiomes on aboveground pathogens may also be mediated by changes in the plant phyllosphere (Gong and Xin 2021, Li et al. 2022), e.g. by affecting microbial competition at the leaf surface (Legein et al. 2020). For entire soil communities, Mursinoff and Tack (2017) found that quantitative resistance of Plantago lanceolata to powdery mildew Podosphaera plantaginis was dependent on the soil microbiome, with slower development of infection and lower pathogen aggressiveness when the host plant was growing with local soil biota versus non-local soil biota. In line with these findings, our study suggests that the composition of the natural soil community matters for the performance of an aboveground pathogen. To investigate the mechanisms behind this, future studies could explore the functional links between soil microbiome compositions, physiological plant responses and severity of pathogen infections in natural settings (Ruiz-Gómez et al. 2019).

The impact of co-occurring aboveground organisms on aphid performance depended on the soil community. The host plant was growing with. With soil microbiome A, aphids performed better when being alone on the host plant compared to when co-occurring with mildew and caterpillars, whereas with the other soil microbiomes, effects were absent (or even reversed). Previous studies have shown that soil microbiomes can indeed impact aphid performance (Bonkowski and Roy 2005, Hol et al. 2010, Kos et al. 2015), though a lack of effects has also been shown (Williams et al. 2014, Karley et al. 2017). Arbuscular mycorrhizal fungi, which are particularly well studied in this context, are commonly found to improve aphid performance, probably due to increased food quality (Koricheva et al. 2009). While previous studies have shown that soil microbiomes can indirectly affect the performance of aboveground insects (Pineda et al. 2010, 2020, Heininen et al. 2018), studies on the impact of soil microbiomes on the interac- tion between co-occurring aboveground organisms are still lacking. However, such knowledge is important given that interactive effects should be expected to occur and are likely to contribute to the context dependency of plant–pathogen–insect interactions. Potentially, soil microbiomes can influence the presence and intensity of resource competition between co-occurring aboveground organisms, depending on the extent to which they provide the host plant with nutrients. Moreover, defensive pathways induced by the plant, as well as its microbial community composition in the phyllosphere, are likely to depend on the combination of soil microbiome and identity of the aboveground organism. Hence, soil microbiomes and aboveground organisms can have a joint impact on plant nutrition, defence and phyllosphere communities (Pineda et al. 2010, Humphrey and Whiteman 2020, Smets and Koskella 2020), and may thus interactively shape the performance of co-occurring aboveground organisms via altering host plant traits, biochemistry and biotic interactions. Our results show that such an interactive effect indeed influenced aphid performance. As mildew performance was only dependent on the soil microbiome, our study also suggests that...
the interactive effect of soil microbiome and co-occurring aboveground organisms on performance may differ among guilds or species. These divergent effects might be caused by differences in the ecology or life history traits of aboveground organisms. For example, aphids can migrate to a different host when present on a plant with a non-beneficial combination of soil community and aboveground organisms, while this is obviously not possible for a powdery mildew colony. In theory, powdery mildew may thus have evolved to better withstand the fluctuation in plant biochemistry induced by other aboveground organisms, which are highly variable in space and time, but remained sensitive to the impact of the soil microbial community, which might be more stable over time (Mundra et al. 2015, Mursinoff and Tack 2017). We encourage future studies to further explore the interactive effects of soil biota and co-occurring aboveground organisms on the performance of members from various guilds in order to uncover the generality of such effects. Also, while it was outside the scope of our current study, uncovering the mechanistic underpinnings that drive the interactive effects between aboveground organisms and soil microbiomes presents an interesting research avenue. Lastly, future experiments could investigate reciprocal effects between multiple aboveground organisms and soil microbial composition via plant–soil feedbacks (Kos et al. 2015, Pineda et al. 2020). These insights will ultimately unravel the role of natural soil microbial communities in shaping the ecology and evolution of plant–pathogen–insect interactions.

Conclusion

Our study showed that soil microbial communities influence the performance of plants and aboveground organisms and also shape the interactions between aboveground organisms, suggesting that soil microbiomes are important drivers of the dynamics of plant-based food webs. Soil microbial compositions are continuously shifting due to environmental changes such as rising temperatures (Classen et al. 2015, Rasmussen et al. 2020) or anthropogenic activities (Bissett et al. 2013). Hence, knowledge about the impact of soil microbiomes on plant food webs is highly relevant to understand and predict potential shifts in the ecological and evolutionary dynamics between plants, pathogens and insects. Furthermore, from an applied perspective, our findings can be used to develop agricultural strategies to control pests and diseases on crops via manipulation of soil microbial communities.

Acknowledgements – We thank Anaís Carpelan for her help with the experimental work.

Funding – This research was supported by a grant from the Swedish Research Council Vetenskapsrådet (2015-03993 to AJMT).

Author contributions

Laura J. A. van Dijk: Conceptualization (equal); Formal analysis (lead); Investigation (lead); Methodology (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). Ahmed Abdelfattah: Formal analysis (supporting); Methodology (supporting); Writing – review and editing (equal). Johan Ehrén: Conceptualization (equal); Supervision (supporting); Writing – review and editing (equal). Ayco J. M. Tack: Conceptualization (equal); Supervision (lead); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.cc2fq64j> (van Dijk et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

References


De Vleesschauwer, D. et al. 2008. Pseudomonas fluorescens WCS374r-induced systemic resistance in rice against Magna-


Feeny, P. P. 1966. Some effects on oak-feeding insects of seasonal changes in the nature of their food. – Univ. of Oxford Press.


