Plant Soil
https://doi.org/10.1007/s11104-025-07419-1

RESEARCH ARTICLE

Differences in nutrient content between heterospecific plant neighbours affect respiration rates of rhizosphere microbiota

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Received: 17 December 2024 / Accepted: 26 March 2025

Abstract

Aims Plant-soil interactions play a pivotal role in governing ecosystem dynamics. Plants directly interact with rhizosphere microorganisms, providing them with labile carbon in exchange for mineral nutrients that are the product of decomposition. Such processes are modulated by ecological interactions between plant species in ways that are not fully understood. We assessed whether rhizosphere respiration rates, as a proxy for decomposition, are influenced by i) heterospecific versus conspecific plant interactions, and whether these effects are positive or negative, and ii) how these effects are linked to the identity and the below- and aboveground functional traits of the interacting plant species.

Methods We conducted a greenhouse experiment with 300 pairwise hetero- and conspecific combinations of ten Mediterranean herbs and shrubs species, covering a range of functional distances calculated based on 33 traits. In addition, we quantified heterotrophic respiration in the rhizosphere as a proxy of decomposition.

Results Plant neighbour identity was the main factor explaining changes on respiration rates. Respiration increased along with the functional distance between heterospecific pairs of interacting plants when considering aboveground or nutritional traits. Morphological and belowground traits were not significant predictors of changes on respiration rates.

Conclusions Interspecific plant-plant interactions lead to faster respiration rates in the rhizosphere as functional distance between neighbours increases. This study provides experimental support that functional trait dissimilarities between heterospecific neighbouring plants promote the rates of organic matter decomposition, showing cascading effects of aboveground interactions on belowground ecosystem processes.

Keywords Organic matter decomposition · Ecosystem function · Plant-plant interaction · Plant-soil interaction · Plant traits · Soil microorganisms

Introduction

The interaction between plants and soil microorganisms plays a crucial role in the structure and functioning of

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Responsible Editor: Edith B. Allen.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-025-07419-1.

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natural communities (Wurst & Ohgushi 2015; De Deyn 2017), regulating essential ecosystem processes such as organic matter decomposition and biogeochemical cycling (Taylor et al. 2009). Plants release organic compounds into the rhizosphere through exudation and rhizodeposition, where they are decomposed by heterotrophic microorganisms mainly through aerobic respiration, partly incorporating them into their biomass and partly releasing carbon into the atmosphere in the form of carbon dioxide (Nguyen 2003; Canarini et al. 2019). In turn, the presence of microorganisms in the rhizospere stimulates root exudation (Přikryl & Vančura 1980). Plants benefit from these processes because microorganisms secrete hydrolytic enzymes into the soil solution, the end-products of which (e.g. phosphorus, nitrate) are indispensable nutrients for plant growth and reproduction (Lambers et al. 2009).

Plant communities are main determinants of the rates of soil heterotrophic respiration, which vary along with community composition (Phoenix et al. 2008), vegetation type (e.g. coniferous vs. broadleaf) (Raich & Tufekcioglu 2000) and litter quantity and quality (Buchmann 2000; Schaefer et al. 2009; Joly et al. 2016). In addition, plant-soil interactions are mediated by root-derived carbon and nutrients, such as root exudates, as well as fine root biomass turnover or root biomass loss derived from mortality (Ma & Chen 2018; Canarini et al. 2019).

The outcome of plant-soil interactions may be altered by the presence of other plants, the identity of neighbours being particularly relevant in microbial-mediated soil functions (Eisenhauer et al. 2010). Turner and Schweitzer (2023) observed that neighbouring plants influenced the focal plant they grew alongside, the effects varying depending on the neighbour's identity. This partly stems from the ability of a plant to modify the trait values of its neighbour, which is related to the functional differences between interacting species (Semchenko et al. 2013; Jimeno-Alda et al. 2025). Such functional differences – or functional distances (FD) – have been shown to have a reflection in belowground systems (Valverde-Barrantes et al. 2013), ultimately explaining microbial-mediated soil ecosystem functions (Gould et al. 2016).

The effect of plant-plant interactions on the functionality of soil microbial communities can be theoretically explained by two ecological mechanisms that underlie species coexistence (Goberna et al. 2016). The first scenario corresponds to the trait dissimilarity mechanism, whereby larger functional distances between plants enhance soil microbial processes through niche complementarity (Fig. 1, left, referred to hereafter as the "trait dissimilarity mechanism"). In support of this scenario, basal respiration has been shown to be promoted in tree mixtures compared with monocultures, several root traits such as root diameter, specific root length or branching intensity being significant predictors of soil microbial activity and biomass (Khlifa et al. 2017). Additionally, an experiment with tree mixtures showed that microbial biomass was promoted under higher levels of plant functional diversity based on aboveground traits such as the specific leaf area and leaf phosphorus (P) and nitrogen (N) contents (Chen et al. 2020). A mesocosm experiment helped delving into the mechanisms that might partly underlie these patterns, by showing that more diverse artificial cocktails of root exudates significantly enhance microbial biomass (Steinauer et al. 2016).

Conversely, the second mechanism is based on relative fitness differences, where a group of plants with traits that confer superior competitive ability outcompetes species lacking those traits (Carmona et al. 2019) (Fig. 1, right; hereafter referred to as the 'trait hierarchy mechanism'). Plants with competitive traits—such as rapid growth, efficient nutrient uptake, or high photosynthetic rates—often produce more biomass, leading to increased deposition of leaf litter, root turnover or exudation into the soil that ultimately serve as organic sources for soil microorganisms (Guyonnet et al. 2018; Cardenas et al. 2021; Wan et al. 2022).

Our study aims to investigate how interspecific plant interactions across a wide range of functional distances affect respiration rates of soil microbiota. We conducted a greenhouse experiment where respiration rates were measured in the rhizosphere of 10 focal species in 300 pairwise combinations. We characterized functional distances between neighbours by considering a broad spectrum of traits, including both above- and belowground morphological and nutritional traits. Finally, we assessed whether rhizosphere respiration rates, as a proxy for decomposition, are influenced by i) heterospecific versus conspecific plant interactions, and whether these effects are positive or negative, and ii) how these effects are linked to the identity and the below- and aboveground functional traits of the interacting plant species. We anticipate that the identity of the species, which reflects

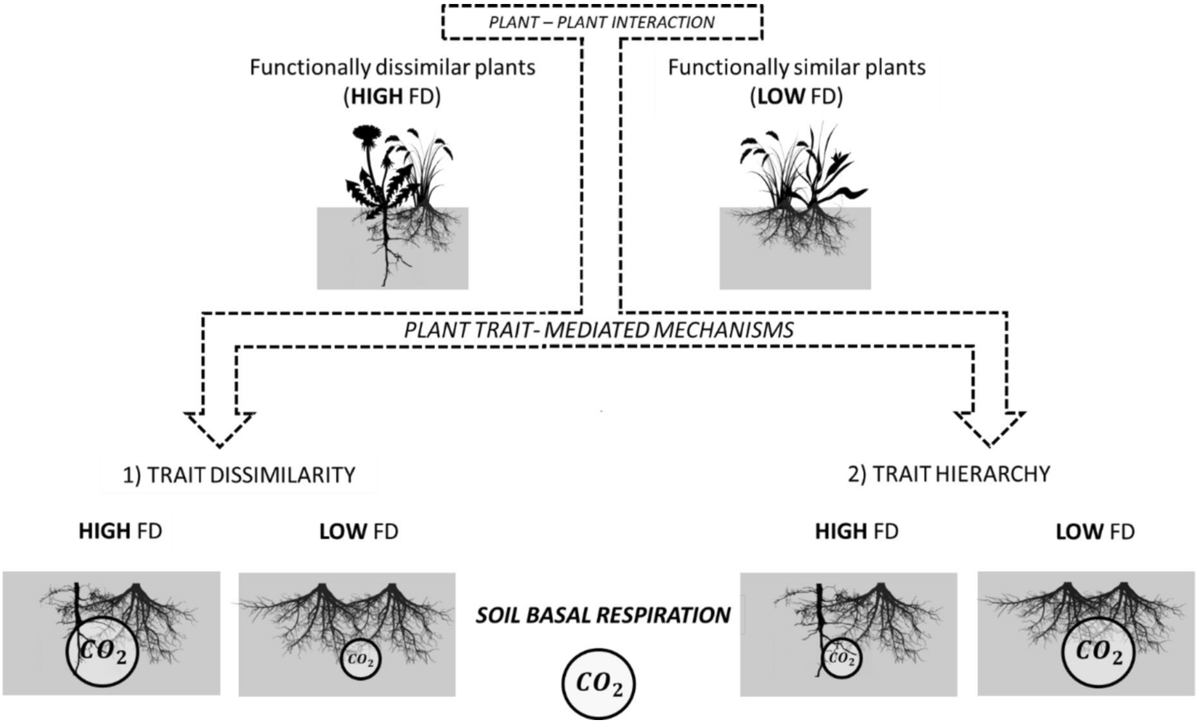

Fig. 1 Schematic representation of the two possible mechanisms (Trait Dissimilarity and Trait hierarchy) explaining how trait-mediated plant-plant interactions can affect plant-soil interactions and microbial ecosystem functions such as soil basal respiration. (BR). Functional Distance between plants (FD) is a multi-trait characterization of the functional differences between the interacting individuals that encompass a broad spectrum of above and belowground morphological and nutrient traits. High values of FD are indicative of functionally dissimilar individuals while low FD values indicate functionally similar individuals. Respiration rates are proportional to the size of the circle containing the CO_2. Under the trait dissimilarity scenario, individuals growing up with dissimilar neighbours (high FD) tend to have larger BR rates. In contrast, under the trait hierarchy mechanisms scenario, BR rates are larger when functionally similar plants (low FD) grow together

their functional traits (including those not measured by us), will primarily explain variations in rhizosphere respiration rates. Previous studies suggest that the impact of plant interactions on the soil microbiome is mediated primarily by the identity of the interacting plants, rather than by other factors such as plant species richness (Eisenhauer et al. 2010). Additionally, functional differences between interacting plants might also influence soil respiration (Valverde-Barrantes et al. 2013) either positively or negatively based on the prevalence of mechanisms of trait dissimilarities or trait hierarchies.

This study provides experimental evidence that heterospecific plant interactions influence rhizosphere respiration rates at the individual level. We measured functional differences of the individuals with a comprehensive set of traits, offering a more detailed phenotypic characterization, that are not usually observed in previous experiments (e.g. Zuo et al. 2016; Chen et al. 2020). Moreover, functional changes are assessed at the individual trait level, accounting for intraspecific variation driven by specific plant-plant interactions and their impact on microbiota rather than the usual species level approach (e.g. Chen & Stark 2000; Turner and Schweitzer 2023).

Material & methods

Plant interaction experiment

To study the impact of plant-plant interactions at varying functional distances (FD) on microbial heterotrophic respiration, we conducted a greenhouse experiment where pairs of individuals from ten species were cultivated on an agricultural soil alongside either heterospecific or conspecific neighbours in various combinations (Fig. S1). After 6 months from February 2021, the experiment finished, and we measured 33 plant traits at the individual level to calculate the FD between every pair of plants growing together. In our previous study (Jimeno-Alda et al. 2025) we analysed the effect of functional distance between plants on plant performance. Our results suggest that trait differences between pairs of interacting plants are reflected in trait dissimilarity mechanisms, while relative fitness differences are represented by trait hierarchy mechanisms and both mechanisms can operate simultaneously during a plant heterospecific interaction, depending on the specific trait measured. For example, for biomass-related traits, focal plants growing with a functionally similar neighbour (low FD values) presented higher trait values, thus better performance. However, root weight trait values were enhanced with the presence of a functionally different neighbour (high FD values) in comparison with the same focal species coexisting with a functionally similar neighbour.

We also quantified the respiration rates from the rhizosphere soil of all individuals. In order to discern the mechanisms (i.e. trait dissimilarities vs. trait hierarchies) explaining the outcomes of interspecific plant interactions on belowground microbial processes, we evaluated the effect of FD on a standardized metrics of basal respiration (BR), that is, the relative change (RC) of BR in heterospecific compared to conspecific interactions.

Study site

We collected seeds of Mediterranean herbs and shrubs from two distinct regions in Spain, Sierra de Guadarrama and Sierra Ministra, respectively characterized by granite and limestone lithologies and a cold semi-arid steppe climate (Bsk, Köppen classification). A total of ten species from diverse evolutionary lineages were selected to encompass a broad spectrum of functional distances. Species included *Trifolium angustifolium* (Fabaceae), *Vicia villosa* (Fabaceae), *Bromus tectorum* (Poaceae), *Hordeum murinum* (Poaceae), *Andryala integrifolia* (Asteraceae), *Helichrysum stoechas* (Asteraceae), *Thymus mastichina* (Lamiaceae), *Micropus erectus* (Asteraceae), *Hirschfeldia incana* (Brassicaceae) and *Plantago coronopus* (Plantaginaceae).

The soil used as the substrate for seeding these species was collected in February 2021 at La Canaleja Agricultural Research Field Station (INIA, CSIC) in Alcalá de Henares, central Spain (40.51, −3.31), a semi-arid area with low and irregular rainfall averaging 353 mm per year. Soils are sandy-loam Calcic Haploxeralfs, have low organic carbon content (7.5 g kg^{-1} in the topsoil), low salinity (0.123 dS m^{-1}), and neutral pH (7.9) (Martín-Lammerding et al. 2015). We sampled soils from a depth of 5–20 cm to minimize the seed bank, sieved it (<5 mm) and heated it at 120 °C for one hour to reduce the microbial load. With this procedure, we aimed to standardize the initial conditions across soil samples ensuring that any differences in soil respiration could be attributed to plant species interactions and not to variations in microbial activity from the original soil communities. Soils were subsequently mixed with silica sand in a 9:1 volume ratio, which we used to fill 5.7-L pots (dimensions $12\times25\times18$ cm) for plant cultivation.

Experimental design

To examine plant interactions, 300 pots were seeded in a greenhouse with two individuals each to include all possible pairwise combinations of the ten species in a fully randomized design. This design resulted in 45 heterospecific pairs and 10 conspecific pairs, each replicated six and three times, respectively, to capture the variability in response to neighbouring plants. In total, 600 plants were grown, with 540 individuals in heterospecific pairings across 270 pots and 60 individuals in conspecific pairings across 30 pots. Due to plant mortality, 50 pots (100 individuals) were excluded from subsequent analyses (see Table S1 and S2 for further information). We selected 2–5 seeds per species sown at 1 cm depth to ensure the germination of at least one individual per species and pot. Seeds were seeded at an equal distance from each other and from the edges of the pots (approx. 5–6 cm). When more than one individual per species emerged, the surplus seedlings were removed, retaining only one individual. The experiment started with the first watering on February 10th, 2021, with pulses of 60 s, five times a day (1.83 ± 0.24 L/m^2 every 5 min). No fertilization was applied.

Plant trait measurement and rhizosphere soil sampling

Plants were harvested and rhizosphere soil sampled between June 22th and July 30th 2021 as individuals reached senescence, thus completing their life cycle. Immediately after harvesting a plant, we measured aboveground traits, separated the roots from the bulk soil to measure belowground traits, and gently shook the roots to collect the soil adhered to them, ensuring no root fragments remained in the sample. Rhizosphere soil samples were immediately frozen at −20 °C until the respiration assay was performed.

Plant morphological, physiological, and reproductive traits were measured on 500 mature individuals (Table S3 and Table S4). After harvesting and sampling, for 234 individuals with sufficient biomass, macro- and micronutrient contents in leaves and roots were also analysed. Leaf and root samples were oven-dried at 60 °C, ground, and analysed for carbon (C) and nitrogen (N) using a LECO TruSpec CN elemental analyser (LECO, USA). Macro- and micronutrient concentrations were determined after digestion in HNO_3 and H_2O_2 with an Ultraclave microwave (Milestone SRL, Milan, Italy) and quantified by ICP-OES (THERMO ICAP 6500 DUO, Thermo Fisher Scientific Inc., USA) (Table S3 and Table S4).

Microbial basal respiration in rhizosphere soils

Out of a total of 500 rhizosphere samples collected, we selected those corresponding to 234 individuals for which we had leaf and root nutrient data and respiration measurements (see Table S5 for further information).

Prior to setting up the incubation assay to measure respiration rates, we quantified soil gravimetric humidity at 105 °C with the aim to standardize the soil water content across samples. We weighed 2 g of rhizosphere soil into 10 ml air-tight containers with silicone caps, and added distilled water to each sample as needed up to a common value of 19% soil gravimetric humidity for all samples (i.e. to force all samples match the humidity value of the sample with the highest water content). We incubated the samples in the dark at 28 °C for 7 days. At the end of the incubation, CO_2-C accumulated in the headspace was extracted by using a syringe and injecting it into an infrared NDIR CO_2 sensor 5700 Headspace Carbon Dioxide Analyser (Illinois instruments, USA). Basal respiration was calculated as the amount of carbon mineralized daily per unit mass of dry (105 ºC) weight soil ($\mathrm{mgC-CO_2kg^{-1}d^{-1}}$).

Statistical analysis

To assess whether soil basal respiration (BR) rates in the rhizosphere of a focal plant species are influenced by the identity of (and functional distance between) neighbouring plants, we calculated the Relative Change (RC) of BR. This metrics captures how BR rates shift when influenced by a heterospecific versus a conspecific neighbour (Eq. 1).

$$RC_{ab} = \frac{BR_{ab} - \overline{BR_{aa}}}{\overline{BR_{aa}}} \tag{1}$$

where RC_{ab} refers to the Relative Change of BR rates provoked by the individual of species *a* when interacting with its neighbour of species *b*; BR_{ab} is the BR of the individual of species *a* growing with the neighbour individual of species *b*, $\overline{\mathrm{BR}_{\mathrm{aa}}}$ is the mean BR of the conspecific combinations of species *a*. We used $\overline{\mathrm{BR}_{\mathrm{aa}}}$ to compare effects derived from a conspecific combination with the effects derived from a heterospecific combination at equivalent densities (pairwise combinations). RC was log-transformed to achieve the normality assumptions in the subsequent statistical models.

Although BR measurements were taken for 234 individuals, only 110 pairs have BR measurements for both individuals that constitute the pair (see Table S1, bottom, for the combinations).

For testing the interspecific differences in RC values we applied a one-way analysis of variance using *aov* function of the package *stats* R v 4.3.2. In these models, the dependent variable was RC, and the independent variable was the species to which the focal individual belonged. We subsequently tested whether RC values for each focal species significantly depart from zero using Student's t-test analyses with the *t.test* function in the R package *stats.* Despite neighbour identity being a key factor affecting respiration rate (RC) values, we performed this test as a first step with this analysis to check for the existence of interspecific differences in RC overall. Then, we added layers of complexity by examining the potential effects of the focal and neighbour species and, finally, the effect of the functional diversity (FD) between them.

The functional distance between pairs of individuals growing together were quantified using the *gowdis* function within the *FD* package for R (Laliberté et al. 2014), which allows considering both quantitative and categorical traits. Gowdis employs the Gower similarity coefficient, described by Podani (1999), and afterwards transforms it into a dissimilarity coefficient. As FD varies with the traits included in its calculation (Sheng et al. 2022), we used different sets which are informative from a morpho-physiological perspective (morphological or nutrient traits), multi-compartment perspective (aboveground or belowground traits), or multi-functional perspective (integrating all the traits mentioned above together with reproductive traits) (See Class 1 and Class 2 columns in Table S3).

To test how trait differences described as FD influence BR, we employed linear mixed-effects regression (LMER) models. We fitted five separate LMERs, each incorporating different FDs calculated with different subsets of traits to specifically assess the influence of trait types on RC values of basal respiration. Analyses were run with the function *lmer* from the *lmerTest* package for R (Kuznetsova et al. 2017). Trait values were log-transformed before calculating FD, which is included in the LMER models. (Eq. 2).

$$RC\left(BR_{focal}\right) \sim FD + (1|focal) + 1(1|neigbour) \quad (2)$$

where RC(BR_{focal}) as response factor, is the Relative Change of the BR rates measured in the focal individual, and FD as explanatory factor is the functional distance between focal and neighbour individuals. The species identities of the focal and neighbour individuals were included as random effects in all models. We included species identity as a random effect to account for variation between focal and neighbor species without emphasizing them in the fixed effect analysis. This approach enables generalization of the functional diversity (FD) effect across species, rather than focusing on individual species. Treating species identity as random allows control of species-specific variation, directing attention to the overall impact of functional distance.

Finally, we tested the relationship between coarse roots, fine roots and root to shoot ratio with rhizosphere basal respiration using the *aov* function in the *lmer* package for R.

Results

Effects of focal plant species identity on microbial respiration

BR ranged from 1.07 to 10.14 $mgC-CO_2kg^{-1}d^{-1}$ across the rhizosphere soils of plant individuals,

Table 1 Mean values ± standard error of rhizosphere basal respiration (BR, expressed in $mgC-CO_2kg^{-1}d^{-1}$) measured for each combination of the ten species of the experiment

FOCAL SPECIES											
		ANIN	BRTE	HEST	HIIN	HOMU	MIER	PLCO	THMA	TRAN	VIVI
NEIGHBOUR SPECIES	ANIN	2.68 ± 0	NA	2.7 ± 0.3	2 ± 0.2	NA	2.7 ± 0.3	NA	2.2 ± 0.4	NA	NA
	BRTE	NA	4.0 ± 0.6	NA	NA	3.6 ± 0.7	NA	2.3 ± 0.4	NA	NA	NA
	HEST	3.0 ± 0.4	NA	2.0 ± 0.4	1.9 ± 0.3	NA	1.2 ± 0.1	1.9 ± 0.3	NA	3.6 ± 1.3	NA
	HIIN	1.3 ± 0.2	NA	1.6 ± 0	2.3 ± 0.2	NA	2 ± 0.4	NA	1.8 ± 0.2	2 ± 0.3	NA
	HOMU	NA	2.6 ± 0.2	NA	NA	3.8 ± 1 86	NA	1.8 ± 0.4	NA	NA	3.2 ± 0.8
	MIER	1.1 ± 0	NA	1.6 ± 0.2	1.6 ± 0.2	NA	1.5 ± 0.2	3.2 ± 0	1.9 ± 0.3	2.7 ± 0.4	2.5 ± 0.8
	PLCO	NA	NA	1.9 ± 0.3	NA	1.7 ± 0.3	2.0 ± 0.1	3.4 ± 0.5	1.8 ± 0.3	3.2 ± 1.1	2.9 ± 0.3
	THMA	3.6 ± 0.9	NA	NA	1.6 ± 0.2	NA	2.8 ± 0.3	2.7 ± 0.7	2.0 ± 0.3	3.4 ± 0.2	3.8 ± 0.6
	TRAN	NA	NA	2 ± 0.2	2.4 ± 0.4	NA	2.5 ± 0.2	2.1 ± 0.3	3 ± 0.6	2.8 ± 0.4	3.6 ± 0.9
	VIVI	1.9 ± 0.3	NA	NA	NA	3.5 ± 0.3	1.9 ± 0.5	2.2 ± 0.3	3.8 ± 1.7	2.3 ± 0.6	5.5 ± 1.4

Total number of individuals which BR was measured was 324 (See Table S5). Acronyms correspond to the name of the species: TRAN *Trifolium angustifolium*; VIVI *Vicia villosa*; BRTE *Bromus tectorum;* HOMU *Hordeum murinum;* ANIN *Andryala integrifolia;* HEST *Helichrysum stoechas;* THMA *Thymus mastichina;* MIER *Micropus erectus;* HIIN *Hirschfeldia incana;* PLCO *Plantago coronopus*

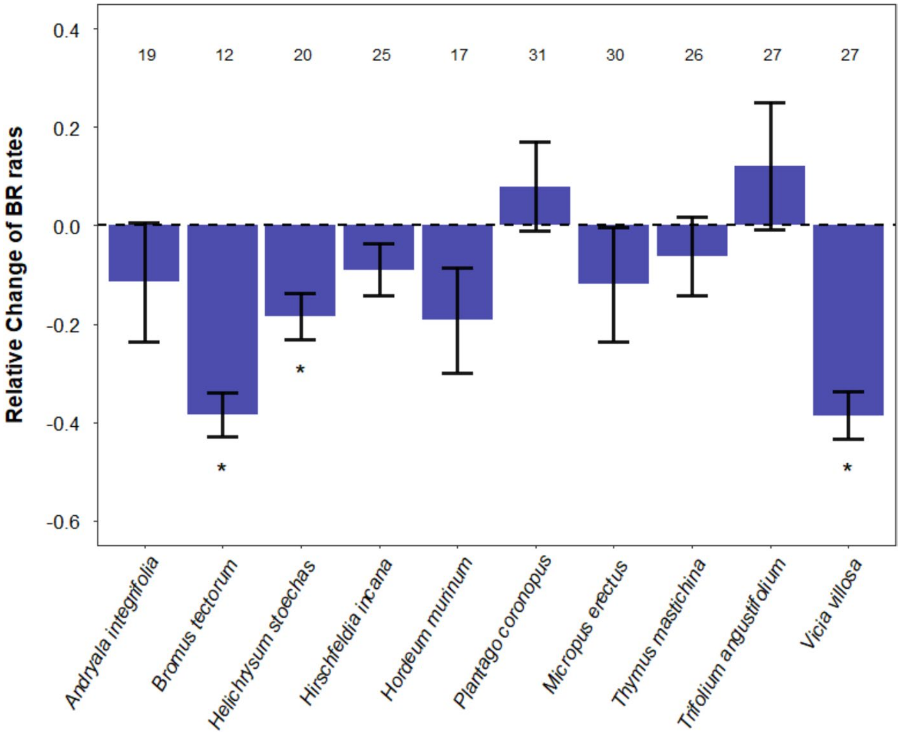

Fig. 2 Mean values ± standard error of the relative change of BR rates experienced by the ten focal species interacting with a heterospecific neighbour ($n = 234$). RC values are averaged across all heterospecific pairs for each species. Negative RC values represent a decrease on BR rates and positive RC values an increase of BR rates due to the neighbour effect. Numbers above bars represent sample size for each species (see Table S5 for further information about the individuals measured). Asterisks (*) indicate that RC effects received by focal species significantly differ from 0 ($p < 0.05$)

while RC values for the focal species ranged from −0.81 to 1.36. Overall, there were marginally significant differences in the RC values of the ten focal species ($F_{9,154} = 1.89$, $p = 0.058$). Most species exhibited lower basal respiration rates in the presence of a heterospecific compared to a conspecific neighbour, although only for three species (*Bromus tectorum*, *Helichrysum stoechas*, and *Vicia villosa*) differences were significant (i.e. RC values departed significantly from zero; Fig. 2; Table 1).

Effects of focal and neighbour plant species identity on microbial respiration

When calculating the RC values for each focal-neighbour combination, we found that the BR rates of focal species increased in 15 heterospecific combinations compared to conspecific ones, while they decreased in 37 heterospecific combinations out of a total 45 heterospecific combinations (Table 2). Interestingly, most species showed both positive and negative responses in RC values depending on the identity of its neighbour (Table 2).

Effects of functional distance between pairs of plant individuals (FD) on microbial respiration

The FD between the focal plant and its neighbour calculated with all 33 traits, did not explain the RC in BR (Table 3, Trait Class All). However, when FD was calculated with either aboveground or nutrient traits (i.e. macro- and micronutrient contents in leaves and roots), a significant and positive relationship emerged between FD and RC (Table 2) indicating that growing with a heterospecific individual differing in these traits promotes BR in the rhizosphere of focal species. However, growing with similar individuals in terms of morphological and belowground traits did not alter BR (Table 3). In all models, the identity of focal and neighbour plant species were significant factors explaining the variance observed in RC values, indicating that: 1. individuals of the same focal species tend to share similar RC values, and 2. individuals of the same neighbour species tend to exert similar effects on focal species (Table 3, Random effect Focal and Random effect neighbour, respectively).

Table 2 Rates of change (RC) of basal respiration (BR) for 10 focal species depending on different neighbour combinations (n = 234)

		FOCAL SPECIES									
		Andryala integrifolia	*Bromus tectorum*	*Hirschfeldia incana*	*Helichrysum stoechas*	*Hordeum murinum*	*Plantago coronopus*	*Micropus erectus*	*Thymus mastichina*	*Trifolium angustifolium*	*Vicia villosa*
NEIGHBOUR SPECIES	*Andryala integrifolia*	-	NA	−0.27	0	NA	NA	0	−0.2	NA	−0.4
	Bromus tectorum	NA	-	NA	NA	−0.11	−0.42	NA	NA	NA	NA
	Hirschfeldia incana	−0.5	NA	-	−0.3	NA	NA	0.31	−0.11	−0.11	NA
	Helichrysum stoechas	0.13	NA	−0.18	-	NA	−0.18	−0.45	NA	0.57	NA
	Hordeum murinum	NA	−0.33	NA	NA	-	−0.53	NA	NA	NA	−0.16
	Plantago coronopus	NA	−0.6	NA	−0.29	−0.55	-	0.34	−0.13	−0.03	−0.48
	Micropus erectus	−0.6	NA	0.07	−0.29	NA	1.15	-	−0.07	0.78	−0.55
	Thymus mastichina	0.35	NA	−0.2	NA	NA	0.34	0.41	-	0.69	−0.32
	Trifolium angustifolium	NA	NA	0.07	−0.14	NA	−0.44	0.66	0.51	-	−0.35
	Vicia villosa	−0.3	NA	NA	NA	0.1	−0.61	−0.66	−0.32	−0.59	-

Negative (positive) values indicate a reduction (increase) of BR rates due to heterospecific neighbour effects. NA indicates missing values due to insufficient amount of sample to measure respiration

Table 3 Linear mixed-effects regression (LMER) models testing the effects of Functional Distance (FD) between pairs of plant individuals on the rate of change (RC) of basal respiration (BR)

Trait class	Estimate ± S.E.	D.F	t-Student	P-value	Random effect: Focal P—value	Random effect: Neighbour P—value	Marginal R^2 / Conditional R^2
All	0.61 ± 0.47	156.32	1.33	0.19	< 0.001	< 0.001	0.009 / 0.269
Aboveground	0.92 ± 0.39	158.03	2.34	0.02	< 0.001	< 0.001	0.027 / 0.294
Belowground	−0.35 ± 0.42	126.86	−0.82	0.41	0.018	0.005	0.005 / 0.253
Nutrient	2.55 ± 1.10	23.12	2.32	0.03	0.03	0.03	0.099 / 0.844
Morphological	0.27 ± 0.43	160.15	0.62	0.54	0.01	< 0.001	0.002 / 0.255

Each row represents a model in which FD has been calculated with all traits, aboveground, belowground, nutrient or morphological traits, as classified in Table S3. The identity of focal and neighbour species was included in the model as random factor

These identity effects were the largest contributors to the variance explained in the models (Table 2, Marginal and Conditional R^2 values). For extended information about results obtained from LMER models see Table S6, Table S7, Table S8.

The relationship between the biomass of coarse versus fine roots and BR showed opposing (yet non-significant) trends (Fig. S2). Coarse roots showed a negative non-significant relationship with BR ($F_{1,71} = 1.37$, $p = 0.25$; $r = -0.13$, $p = 0.25$), while that of fine roots was positive and marginally significant ($F_{1,71} = 3.06$, $p = 0.085$; $r = 0.11$, $p = 0.16$) (Fig. S2). Additionally, root to shoot ratio showed a positive non-significant relationship with BR ($F_{1,47} = 4.38$, $p = 0.25$; $r = -0.13$, $p = 0.071$) (Fig. S2).

Discussion

Our experiment examined the effects of interspecific plant-plant interactions on the rates of heterotrophic respiration of microbial communities thriving in the rhizosphere. We found that plant species identity of both focal and neighbour individuals were the main factors explaining respiration rates. In addition, the functional difference between interacting individuals in terms of aboveground traits and nutritional traits enhanced respiration rates, adding experimental support to the scenario according to which trait dissimilarities between neighbouring plant species promote microbial-driven ecosystem processes as discussed below.

Respiration rates changed depending on the focal species with which rhizosphere microorganisms interacted, supporting species-specific effects of plants on microbial respiration (Khlifa et al. 2017). Our results from different focal-neighbour combinations also showed species-specific dependencies, as the magnitude and sign of respiration rates were strongly dependent on the neighbour identity. For example, during the experiment *Plantago coronopus* showed a decrease in RC(BR) rates (−0.53, Table 2, column 6), while *Trifolium angustifolium* exhibited the greatest increase (0.78, Table 2 column 9) because of the presence of a heterospecific neighbour. Moreover, *Trifolium angustifolium* presented the most positive neighbour effect (0.66, Table 2), whereas *Vicia villosa* exerted the most negative neighbour effect on respiration rates (−0.66, Table 2). However, these overall values do not necessarily indicate which species had the greatest influence on others or which was most affected by the presence of a neighbour, as this effect varies between species pairs. The mechanism behind this specificity involves neighbour effects altering the traits of the focal plant, which indirectly influences its interactions with other taxa, such as soil microorganisms (Underwood et al. 2014). Altogether, these results emphasize the crucial role of plant interactions in altering plant traits, showing that these effects extend beyond the plants to the surrounding rhizosphere, ultimately influencing soil microorganisms and microbially-mediated ecosystem functions (Kardol et al. 2013; Wurst & Ohgushi 2015). It becomes evident that comprehending the mechanisms underlying these species-specific dependencies requires a thorough examination of the traits inherent to each species involved in the interaction.

Our results did not reveal a significant effect of plant functional distance on respiration rates when all traits were considered together. This may be due to the fact that the combination of different trait types (e.g., morphological, nutritional, above- and belowground traits) can mask the effect of individual traits, since each trait may play a distinct role in plant-soil microbial interactions (Chen et al. 2020). However, when the functional distance between interacting individuals was calculated based on the plant contents in total carbon, macro- and micronutrients in their leaves and roots we detected a significant effect of functional distance on respiration rates. This finding is consistent with several previous studies. Joly et al. (2016) demonstrated that variations in nutrient concentrations in litter leachates can determine soil microbial respiration, as leachates of broadleaf litter containing higher amounts of carbon and nitrogen than those of conifers produced longer-lasting effects on microbial respiration. Leaf N content is highly related to leaf CO_2 assimilation rates (Sinclair & Hory 1989) and net photosynthesis (Reich et al. 1998). A relevant proportion of C fixed during photosynthesis in aboveground tissues is exported belowground, and part of root C ends up entering the soil solution via exudation and rhizodeposition (Farrar & Jones 2000; Nguyen 2003). Also, the contents of fine root N are directly associated with root exudation (Sun et al. 2020), and so root N correlates with soil fungal and bacterial biomass as high levels of nitrogen inputs promote decomposition rates and nutrient transference from plants to soil microorganisms (De Long et al. 2019). Such relationships are, however, not always obvious depending on the ecosystem considered, as Ferlian et al. (2017) showed when analysing the effect of leaf and fine root N and C contents and ratios of over 30 temperate tree species on soil microbial biomass and respiration. Here, we argue that the positive impact of functional dissimilarity in nutrient contents between interacting plants on rhizosphere respiration rates suggests the relevance of resource complementarity in determining microbial responses. The same mechanism can be invoked to explain the positive impact of functional dissimilarity in experimental exudate cocktails (Steinauer et al. 2016) or leachate nutrient traits (Joly et al. 2016) on soil microbial responses.

The functional distance in aboveground traits between interacting species exhibited a subtle yet significant influence on basal respiration in the rhizosphere. These patterns were primarily driven by leaf and shoot nutrient contents, and secondarily by leaf and shoot morphological traits (Table S6, Table S7). In our case, leaves and shoots exhibited higher contents of C, N and P, compared to the roots of the same individual (Table S8). Furthermore, the weight of leaf and shoot tissues was significantly larger than that of root tissues (Table S8). Additionally, root to shoot ratio presented a slightly negative and marginally significant influence of basal respiration (Figure S2, c). Leaf and shoot traits might have influenced rhizosphere respiration rates through the mechanisms discussed above. In addition, plants may resorb carbon and nutrients from senescent aboveground tissues to new sink tissues and may be redistributed among living tissues (Brant & Chen 2015) and finally released into the soil. This would result in an increased contribution of nutrients from plants to the soil, thereby enhancing rhizosphere respiration rates.

Contrary to our expectations, we did not find a significant influence of belowground traits on soil respiration rates. These traits are expected to exert a strong influence on soil functions due to its close contact with the rhizosphere (Wurst & Ohgushi 2015). Variations in root architecture, size and exudation rate can be linked to the plant ecological strategy (Reich et al. 2008; Guyonnet et al. 2018). While fine roots are associated with an acquisitive strategy that relies on fast growth and reproductive activity, coarse roots associate with a conservative strategy that is based on the production of accumulative and structural structures (Sun et al. 2020; Navarro-Cano et al. 2024). Root exudates, which play a key role in stimulating microbial activity are secreted primarily from fine root tips (Guyonnet et al. 2018; Canarini et al. 2019), thus differential root traits may have various impacts on ecological processes. This was partly reflected in our results, as we obtained a marginal positive influence of the biomass of fine roots on rhizhospheric respiration, but a marginally negative (yet non-significant) influence of coarse roots and root to shoot ratio (Fig. S2). These opposing trends possibly masked the overall influence of belowground traits, although the correlations found were weak.

Lastly, the predictive strength for most of our models is primarily driven by the variable plant identity, with a smaller contribution from the variable functional distance between the interacting plants.

Despite its subtle influence, it remains significant, as plant functional diversity is a key factor mediating plant–soil microorganism feedback, which in turn influence ecosystem processes (e.g., Sardans 2013; Kardol et al. 2013; Lange et al. 2014). This leaves room to include other relevant factors influencing plant-soil interactions. For instance, harsh environmental conditions can intensify positive relationships, thereby clarifying trends and effects (see Navarro-Cano et al. 2016). Thus, changing the experimental conditions towards more stressful conditions could shed light on other plant-microbial relationships. Furthermore, incorporating measurements of fine root turnover, quantifying the utilization of exudates as biomass sources by soil microorganisms, and determining the amount of carbon consumed and incorporated into microbial biomass would help further elucidate the mechanisms underlying our results.

Conclusions

Our extensive phenotyping of individual plants interacting with heterospecific neighbours highlights how functional differences driven by both intraspecific and interspecific trait variability, promote microbial-driven processes in the rhizosphere. This contributes to the growing body of evidence linking plant traits with soil microbiota (e.g., Buchmann et al. 2000; Bardgett et al. 2014; De Deyn 2017; De Long et al. 2019) and giving support to the idea that ecological interactions among primary producers regulate ecosystem processes controlled by decomposers.

Acknowledgements We are most grateful to David San Martín, Patricia Plaza, Raúl San Juan, and the staff at La Canaleja Research Station (INIA-CSIC) for their valuable help in setting up the assays, monitoring plant development and quantifying plant traits. Furthermore, we are also grateful to Daniel Rodríguez and Santiago Donat from Centro de Investigaciones Sobre Desertificación (CIDE, CSIC-UV-GV), for their great help in the laboratory.

Author Contributions M.V., J.A.N.C. and M.G. acquired funds and designed the experiment; J.J.A., J.A.N.C. and M.G. collected plant and soil material, carried out the experiment and collected the data; J.J.A. and M.V. performed the statistical analyses and wrote the first draft, and all authors edited the manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. The authors received grants PID2020-119634 GB-I00 and PID2020-113157 GB-I00 funded by MCIN/AEI/https://doi.org/10.13039/501100011036 and CIPROM/2021/63 from Generalitat Valenciana. J.A.N.C. received grant RYC2019-026848-I also funded by MCIN/AEI and by "ESF Investing in your future". J.J.A. was financially supported by the Ministry of Science and Innovation (FPI grant 18/085747).

Data Availability Data and the R code to run all the analyses are available as Supplementary Information.

Declarations

Competing Interests The authors have no conflicts of interest to declare.

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