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# **Soil microbes drive the effect of plant species and genotypic diversity interaction on productivity.**

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## **Abstract**

The effect of plant species and genotypic diversity on productivity has been well documented but little is known about the contribution of the interaction between species and genotypic diversity. Since the influence of soil microorganisms on the plant diversity-productivity relationship is increasingly recognized, we investigated potential interactions between plant species diversity, plant genotypic diversity and soil microbes. We hypothesized that the non-additive effects of plant species and genotypic diversity on productivity could be microbe-driven.

We set up pea and wheat monocultures and mixtures in a growth chamber, varying for their levels of genotypic diversity under three different soil microbial contexts. We assessed plant shoot and root biomass production, soil mineral nitrogen content and described the soil bacterial communities.

We found that shoot biomass in mixtures involving both species and genotypic diversity was higher than expected considering the additive effects of plant species diversity and genotypic diversity. Rather than a synergy between two positive effects of species and genotypic diversity, we observed that species mixture compensated the negative effect of genotypic diversity. Regarding microbial influence, we found that the effect of plant species diversity, plant genotypic diversity and their interaction on productivity were all driven by the soil microbial community as no effect was observed in a pre-sterilised soil.

Our study suggests that the plant diversity-productivity relationship could be shaped by a three-way interaction between plant species diversity, plant genotypic diversity and soil microbes. Thus, plant-microbe and plant-plant interactions could be a determinant of the plant diversity-productivity relationship.

**Keywords** diversity-productivity relationship, genotypic diversity, plant-microbe interactions, soil microbes, species diversity

## Introduction

Plant species diversity is known to have positive effects on ecosystem functioning. In particular, a lot of studies highlighted a positive effect of species diversity on productivity (Tilman et al. 1996; Hooper and Vitousek 1997; Yachi and Loreau 1999; Hector et al. 1999) with evidence that functional group richness, and further, functional dissimilarity between groups (Heemsbergen et al. 2004), play a greater role than species richness per se (Grime 1997; Balvanera et al. 2006). The mechanisms that could explain a positive diversity-productivity relationship are often grouped under two main categories of effects: “complementarity effects” (i.e. resource partitioning and/or facilitation) and “sampling effect” (increase in the probability of including a highly productive species with the increase in the number of species in a community; Loreau et al. 2001; Cardinale et al. 2002). Complementarity effects, and more particularly complementarity in resource use, have received attention as they are potential levers for enhancing the use of available resources in soils and decreasing nutrient losses (Tilman et al. 1996). A recent study highlighted that peak overyielding in a five-species mixture (i.e. gain in productivity as compared to mean monoculture performance) co-occurred with an enhanced resource-use efficiency for water, nitrogen and light (resource use related to dry mass production; Mason et al. 2020).

The same mechanisms can be involved in positive effects of within-species genotypic diversity on productivity (Hughes et al. 2008). Indeed, although genotypic variability within a plant species is supposed to be lower than between species variability (Jung et al. 2010; Albert et al. 2011; Kichenin et al. 2013), many studies have shown a positive effect of increasing within- species genotypic diversity on productivity (Johnson et al. 2005; Crutsinger 2006; Cook-Patton et al. 2011). Cook-Patton et al. (2011) showed that increasing the genotypic diversity in an herbaceous species (from one to eight genotypes) gave rise to a similar increase in productivity (about 17%) than increasing species diversity (from one to eight old-field species excluding the previously studied herbaceous species). But, in another study, Prieto et al. (2015) showed that increasing the species diversity (from one to five grassland species) increased productivity, whereas increasing the genotypic diversity within each species of the mixture (from one to ten genotypes per species) did not influence plant productivity.

To go further, there is evidence that species and genotypic diversity can interact and lead to modifications in competition and dominance patterns among species (Fridley and Grime 2010; Crawford and Rudgers 2012; Schöb et al. 2015). As an example, Fridley and Grime (2010) showed with neighborhood modeling that an increase in genotypic diversity in grassland communities lead to a reduction in interspecific competition which tended to increase community effective richness (i.e. a higher number of species was maintained over the experiment, with a dependence on soil depth). In this case, this change in community structure was not large enough to induce a change in productivity. But, in other studies, it has been shown that increasing genotypic diversity within a plant species can either increase the benefit of species diversity on productivity (Crawford and Rudgers 2012) or decrease it (Schöb et al. 2015). Thus, understanding the interaction between species and genotypic diversity is mandatory to understand the plant diversity-productivity relationship.

Soil microorganisms can also greatly influence plant primary productivity, through direct (mutualists and pathogens) or indirect effects (free-living microorganisms through their influence on resource availability; van der Heijden et al. 2008). The effects of microbial mutualists, in particular of rhizobia and arbuscular mycorrhizal fungi, and of pathogens on plant productivity are well-studied and there is evidence for strong effects of the microbial species identity (Vogelsang et al. 2006; Wagg et al. 2011b; Bever et al. 2013). Soil microbes can also affect plant community composition and functioning (Reynolds et al. 2003). One well-studied mechanism is plant-soil feedback (van der Putten et al. 2013) which can occur through the influence of plants on the soil microbial community which, in turn, influences the future plant community (Bennett and Klironomos 2019). Indeed, the presence of a particular plant species can promote certain soil microbes (e.g. mutualistic partners or pathogens; Mills and Bever 1998; Bever 2002) and, due to the specificity of the interactions between plants and soil microbes (i.e. the existence or not of an interaction and the partner response to the interaction), this could favor or reduce the growth of the seedlings of this species. The existence of a negative plant-soil feedback could be a promoter of plant community diversity while a positive plant-soil feedback could give rise to the dominance of a species (Revillini et al. 2016).

The interdependence between plants and microorganisms for essential functions (e.g. nutrition) can be so strong that it could be useful to consider a plant and its associated microbes as a single functional and evolutionary unit, referred to as a holobiont (Zilber-Rosenberg and Rosenberg 2008; Vandenkoornhuyse et al. 2015). This is of particular interest in the study of the plant diversity-productivity relationship because it suggests that microbes could play a significant role in explaining this relationship. Many studies have highlighted the crucial role of plant pathogens and mutualists, especially fungi, in explaining either the existence (Maron et al. 2011; Wang et al. 2019) or the shape (Klironomos et al. 2000; Schnitzer et al. 2011) of the plant diversity-productivity relationship. These studies were focused on the link between plant species richness and productivity. But to the best of our knowledge, there is no study to date that investigated the effects of soil microbes on i) the link between plant genotypic diversity and productivity and ii) the interactions between plant species and genotypic diversity on productivity.

In this study, we wanted to determine if the effect of the interaction between species and genotypic diversity on productivity was influenced by the soil microbial community. Our experimental design involved pea- wheat mixtures varying for their levels of genotypic diversity in three soil microbial contexts (sterilised soil, native microbial community, exogenous microbial community). We first hypothesized that combining plant species and genotypic diversity could lead to non-additive effects on ecosystem productivity and, more specifically, synergistic effects could arise through an increase in complementarity and resource use as compared with a case involving only one of the diversity levels. Then, we hypothesized that soil microorganisms could drive potential interactions between plant species and genotypic diversity based on the assumption that the effect of soil microbes on plant productivity could be genotype and/or species dependent and thus lead to changes in plant-

plant interactions (e.g. competition enhancement or reduction). We expected the diversity effects on plants to be lower in a soil with low microbial diversity (i.e. sterilised soil) and different between a native and an exogenous microbial community due to differences in plant-soil feedbacks (Bennett and Klironomos 2019). Reciprocally, we also expected an effect of the plant community on soil microbial community structure and diversity as already shown in monoculture-mixture experiments using pea and wheat (Taschen et al. 2017).

## **Materials and methods**

### *Experimental design*

We grew two species which are commonly grown in mixtures: wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.), and for which positive plant-plant interactions have been described (Duchene et al. 2017). Three genotypes of each species were selected with contrasting phenotypic characteristics (described in Table S1). Genotypic monocultures and genotypic mixtures were set up in species monoculture or species mixture resulting in four species monoculture treatments: one wheat genotype (W1), one pea genotype (P1), three wheat genotypes (W3), three pea genotypes (P3); and four pea-wheat mixture treatments (W1P1, W1P3, W3P1, W3P3; see Figure 1). W1 refers to the wheat genotype “Flamenko”, W3 refers to the association of the wheat genotypes “Flamenko”, “Renan” and “RE13088”, P1 refers to the pea genotype “Cameor” and P3 refers to the association of the pea genotypes “Cameor”, “Champagne” and “336.11”. Note that the genotypes “Renan”, “RE13088”, “Champagne” and “336.11” were not grown in monoculture due to technical constraints (growth chamber capacity). The analyses were therefore adapted to take this limitation into account (see the “Biomass deviation from additivity” section). The genotype that was chosen for being grown in monoculture for each species was the best performing in our experimental conditions (i.e. higher germination rate and emergence after transplantation) based on the results of a pre-experiment. The abundance was set to six plants per pot whatever the plant diversity and seedlings were planted in circle alternating genotypes and/or species, to ensure each individual had potentially the same neighborhood as the others. Each plant diversity treatment was repeated five times per microbial context (three different soil microbial communities, see “Microbial context” section) as well as a control treatment without plant (control, C), for a total of 135 pots.

### *Plant materials*

Wheat seeds were obtained from Agri Obtentions (Guyancourt, France) and pea seeds from “Centre de Ressources Génétiques” (INRAE Dijon, France). Wheat seeds were placed on blotting paper soaked with osmotic water for six days (one day at 4 °C in the dark, three days at 18 °C in the dark, two days at 20 °C in constant light). Pea seeds were covered with osmotic water for two days (24 °C, in the dark). Wheat and pea were transplanted simultaneously; the first leaf emerged from the coleoptile in wheat seedlings and the radicle emerged from the pea seed. The transplantation time after sowing was

determined according to a pre-experiment to avoid the dominance of one species from the very beginning of the experiment. Transplantation occurred 48 h after pot filling.

### *Microbial context*

The soil used in this experiment was a sieved and gamma-sterilised (Ionisos, France) sandy soil from a natural meadow (CEREEP research station, Saint-Pierre-lès-Nemours, France), with the following characteristics: total organic carbon content: 14.7 g.kg<sup>-1</sup>; total nitrogen content: 1.19 g.kg<sup>-1</sup>; pH: 5.22; CEC: 4.08 cmol.kg<sup>-1</sup>; texture: 6.9% clay, 19.0% silt, 74.1% sand. We wanted to test for an effect of the soil microbial context on the plant community. Thus, in order to avoid a possible confusing effect of soil characteristics, we used the same soil across the different microbial contexts and we inoculated, or not, a microbial community in this soil. The first microbial context, S for sterilised, corresponded to the sterilised soil without inoculation. In microbial context N (native), we inoculated a microbial community that came from the non-sterilised sandy soil cultivated in pea/wheat mixture for three weeks in the laboratory. In microbial context E (exogenous), the microbial community came from a field grown with pea/ wheat mixture (Époisses experimental station, INRAE, Bretenière, France). Six hundred ml of both inoculums were obtained by crushing four times 50 g of sieved soil in a blender for 3x30s with 150 ml of osmotic water (Philippot et al. 2013). The suspensions were then diluted ten times in osmotic water to reach a sufficient volume for the inoculation of the soil.

### *Growth conditions*

Plants were grown in a climatic chamber for 22 days in one-liter pots filled with sandy soil. They were first watered with either 220 ml of osmotic water (S) or 120 ml of osmotic water and 100 ml of inoculum (N and E) which corresponded to 80% of the field capacity. Throughout the experiment, soil moisture was maintained between 60 and 90% of the field capacity, by watering daily with a fixed amount of water and re-adjusting water content after weighing twice a week. The conditions in the climatic chamber were: 70%- hygrometry, 12 h-photoperiod, 20 °C during the day and 18 °C at night.

### *Productivity measurement, soil nitrogen content assessment and microbial community analysis*

Plants were harvested after 22 days of growth; shoots and roots were collected (roots were washed), dried at 50 °C for 48 h and weighed. Shoot biomass was measured for each individual separately whereas root biomass was characterised at the pot scale.

Soil mineral nitrogen content (nitrate and ammonium) at 22 days was measured by shaking 10 g of soil in 50 ml of KCl for 1 h, decantation for 45 min and analysis of the filtered supernatant with the analyser Global 240 (BPC Biosed, Rome, Italy).

Considering the colonization of the pots by plant root systems, we could hypothesize that the soil was under the influence of the plants in the entire pot. Thus, the soil of each pot was mixed and DNA was extracted from a sample of 280 mg (DNeasy PowerSoil Kit, Qiagen, Venlo, Netherlands).

After ensuring that there was no PCR inhibition, 16S rRNA gene was amplified using the Pro341F/Pro805R couple of primers (targeting Bacteria, Takahashi et al. 2014). A second PCR allowed the barcoding of the samples by hybridization of specific couples of primers on the adapters carried by the Pro341F/Pro805R primers. PCR products were normalized (SequalPrep, ThermoFisher Scientific, Waltham, USA), pooled and sequenced on Illumina MiSeq (Microsynth, Balgach, Switzerland).

### *Bioinformatics analysis*

Sequencing data was analysed following Goodrich et al. (2014) recommendations for 16S rRNA gene. Forward and reverse sequences were paired using PEAR (Zhang et al. 2014). Unassembled sequences and sequences smaller than 400 base pairs were discarded as well as chimeric sequences which were detected by reference OTU-picking against the Greengenes Gold database (VSEARCH; Rognes et al. 2016). OTU clustering was done at the 94% identity threshold (determined from the results obtained on a mock community of known species number). Taxonomy was assigned with Greengenes' representative set of sequences and based on the longest sequence of each OTU (highest base pair number). The number of sequences per sample ranged from 22,175 to 78,739. We worked on a rarefied OTU table at the level of 30,000 sequences per sample which excluded one sample from the dataset.  $\alpha$ -diversity indices (number of observed species per sample, Simpson's reciprocal index, phylogenetic diversity) were the mean of ten independent calculations in QIIME (Caporaso et al. 2010).  $\beta$ -diversity (difference in community composition between samples) was obtained by the construction of a Bray-Curtis dissimilarity matrix from the rarefied OTU table.

### *Biomass deviation from additivity*

Diversity effects are usually defined as the difference between the observed yield in mixtures and the expected yield based on the average monoculture performances (Loreau and Hector 2001). As we did not grow all the monocultures, we defined the diversity effects as follows: the effect of genotypic diversity (GE for genotypic effect) in a given species was calculated as the difference in biomass in genotypic mixtures as compared to genotypic monocultures (i.e.  $W3 - W1$  or  $P3 - P1$ ). The effect of species diversity (SE) was the difference in biomass in the pea-wheat mixture as compared to the best of the two monocultures present in our design (i.e.  $W1P1 - W1$ ), in line with what is done to study transgressive overyielding (Trenbath 1974). The effect of combining species and genotypic diversity (SGE) was calculated as the difference in biomass in mixtures involving both diversity levels (i.e.  $W1P3$ ,  $W3P1$  or  $W3P3$ ) as compared with the best monoculture ( $W1$ ). Mean effects and the associated 95% confidence intervals were obtained by bootstrapping (1000 calculations of each difference with replacement at each sampling event) and are showed in Table 1.

We determined if the effect of combining species and genotypic diversity (SGE) differed from the sum of the effect of species diversity (SE) on the one side and genotypic diversity (GE) on the other side, by calculating a "deviation from expected value" (Loreau and Hector 2001; Johnson et al. 2005).

There is a deviation from additivity if “the total response of a variable is greater or less than the sum of the partitioned responses generated by the individual constituents” (Genung et al. 2010). We calculated this deviation as follows:

$$\Delta_{additivity_{ij}} = SGE_{ij} - (SE_j + GE_j)$$

With:

- $\Delta_{additivity_{ij}}$  the deviation from the additivity hypothesis in pot  $i$  in microbial context  $j$ ,
- $SGE_{ij}$  the effect of combining species and genotypic diversity in pot  $i$  in microbial context  $j$  i.e. the observed biomass in pot  $i$  in microbial context  $j$  minus the mean biomass in wheat genotypic monocultures in microbial context  $j$ .
- $SE_j$  the mean effect of species diversity in microbial context  $j$ ,
- $GE_j$  the mean effect of genotypic diversity in microbial context  $j$ .

The expected GE depended on the mixture: the expected GE in W1P3 was the one attributed to pea (i.e. P3-P1), in W3P1 it was the one attributed to wheat (i.e. W3-W1) and in W3P3, the expected effect was the sum of the two effects previously mentioned. Note that this calculation method is based on the hypothesis that the genotypic diversity effect was constant regardless of the number of individual plants involved. We did not consider that the effect of genotypic diversity increased linearly with the number of individuals in a mixture, as this supposed the absence of interaction effects among the individuals of a species.

### *Statistical analyses*

Three pots over the 135 were removed from the dataset as a mistake occurred during the transplantation phase (one genotype instead of another). Fifteen pots on the remaining 132 presented an absence of growth of one of the six plants and three pots presented an absence of growth of two of the six plants. It represented 15.4% of the pots containing plants (5.1, 6.8 and 3.4% for S, N and E respectively). These 18 pots were included in the dataset to keep correct sample sizes (note that the analysis without these 18 pots gave consistent results).

Plant biomass, biomass deviation from additivity, soil mineral nitrogen content and  $\alpha$ -diversity in the bacterial communities (observed species, phylogenetic diversity and Simpson's reciprocal index) were submitted to analyses of variance (ANOVA, type II sums of squares) including the plant treatment, the microbial context and their interaction as explanatory variables. Plant treatment refers either to plant diversity (i.e. a variable including the eight plant diversity treatments; note that for nitrogen content and  $\alpha$ -diversity the control without plant was also included) or mixture diversity (i.e. a variable including the three plant diversity treatments involving both species and genotypic diversity) depending on the model. Wheat and pea per capita shoot biomass was submitted to analyses of variance with the species diversity (species monoculture or species mixture), the genotype, the microbial context and their interactions as explanatory variables. Each analysis of variance was followed by a post hoc Tukey's test.



The  $\beta$ -diversity of the soil bacterial communities was analysed performing a PERMANOVA (including the plant diversity, the microbial context and their interaction as explanatory variables) with Bray-Curtis dissimilarity index, followed by pairwise comparisons. Data were visualised with a non-metric multidimensional scaling (NMDS).

We analysed the effect of soil bacterial richness on plant biomass with a linear model of plant total biomass explained by the microbial context, the plant species (pea, wheat or both), the bacterial community richness and their interactions. The slopes of the regression lines and the associated 95% confidence intervals were calculated holding the microbial context and the plant species effects constant on their average value.

One correlation network per microbial context was built based on the unrarefied OTU table of 16S rRNA gene sequencing. The aim of this analysis was to find statistical associations between pairs of OTU based on the abundance (i.e. sequence counts) obtained from sequencing data. These statistical associations (i.e. partial correlations which are represented by edges in the network) indicate ecological interactions (either positive or negative) between the OTUs (represented by nodes) that we supposed to depend on the soil microbial context. Only the partial correlations higher than 0.075 in absolute value were kept for the representation for readability (note that it gave a good representation of the effect of the microbial context as compared to the networks that included all of the partial correlations). To build the networks, the OTU table was filtered as follows: samples with less than 30,000 sequences were discarded as well as OTUs that represented less than 0.5% of the sequences. An abundance matrix was obtained from the filtered OTU table and described with a Poisson log-normal model (Chiquet et al. 2019) including an offset term to take the differences in sequencing depth between samples into account (the offset of a sample corresponded to the logarithm of the total number of counts in this sample). The best model was selected with a Stability Approach to Regularization Selection (StARS; Liu et al. 2010).

All the analyses were performed with R software version 3.6.1 and the following packages: car for ANOVAs (Fox and Weisberg 2019), agricolae for Tukey's tests (de Mendiburu 2020), vegan for PERMANOVA and NMDS (Oksanen et al. 2018) and mctoolsr for pairwise comparisons (Leff 2017), stats for linear models, t-tests and correlation tests, emmeans (Lenth 2021) and sjPlot (Lüdecke 2020) for regression slope description, PLNmodels for network analysis (Chiquet et al. 2019). Correlation networks were visualised with Cytoscape (Shannon et al. 2003).

## Results

### *Plant community diversity effect on plant biomass production*

Overall, we registered overyielding but not transgressive overyielding, that is, total biomass production increased by 11% in pea-wheat mixtures as compared to species monocultures (Figure 2a;  $t = -5.2$ ,  $p_{df=115} = 1.1 \times 10^{-6}$ ) but, the best mixture (W3P1;  $1.35 \pm 0.13$  g) was not significantly more productive than the best of the two grown monocultures (W1;  $1.22 \pm 0.10$  g; Figure 2b). The increase in biomass in mixtures was mainly due to an increase in shoot biomass in mixtures (+13%;  $p_{df=115} = 2.2$

$\times 10^{-7}$ ) as there was no change in root biomass production ( $p_{df=115} = 0.23$ ; Figure 2a). There was a negative effect of genotypic diversity on total biomass production ( $-6\%$  in genotypic mixtures as compared to genotypic monocultures; Figure 2a;  $t = 2.0$ ;  $p_{df=56} = 0.048$ ) which, as for species diversity effect, occurred through a change in shoot biomass ( $-8\%$ ;  $p_{df=56} = 0.023$ ; no change in root biomass:  $p_{df=56} = 0.93$ ). There was no significant effect of increasing genotypic diversity on total biomass production once wheat and pea were considered separately (i.e. biomass in W3 and P3 did not differ from those of W1 and P1 respectively; Figure 2b).

The deviation of biomass from the additivity hypothesis, i.e. the existence of interaction effects between the two plant diversity levels, was not influenced by the interaction between the mixture diversity and the soil microbial context whatever the plant part (shoots, roots or total; Table S2), therefore we evaluated both effects separately. There was no effect of the mixture diversity on shoot biomass deviation from additivity as all the mixtures (W1P3, W3P1 and W3P3) showed a similar gain in shoot biomass as compared to what was expected under the additivity hypothesis (Figure 2c). However, this gain in shoot biomass was significant in W3P1 and W3P3 ( $+0.07 \pm 0.10$  g and  $+0.07 \pm 0.11$  g respectively i.e.  $+7.2$  and  $+8.4\%$ ) but not in W1P3 ( $+0.04 \pm 0.15$  g i.e.  $+4.6\%$ ; Figure 2c). Unlike shoot biomass, the direction of root biomass deviation from additivity depended on the mixture diversity ( $p_{df=2} = 6.2 \times 10^{-4}$ ; Table S2; negative deviation values for W1P3 and W3P3 and positive deviation value for W3P1; see Figure S1). Overall, shoot and root biomass deviation from additivity were positively correlated (two-sided Pearson's correlation test,  $r = 0.42$ ,  $p_{df=43} = 4.3 \times 10^{-3}$ ).

#### *Differences in soil bacterial community between the microbial contexts*

The three microbial contexts differed in terms of bacterial community structure ( $p_{df=2} = 1.0 \times 10^{-3}$ ;  $R^2 = 0.59$ ; Table S3; Figure S2a and b) and in terms of number of observed species, phylogenetic diversity and Simpson's reciprocal index (lower values in S than N and E and differences in N and E for the three indices; Table S4). An analysis based on correlation networks revealed a similar network structure in the native and exogenous communities with similar numbers of nodes and edges (47 nodes, 63 edges in the native community and 54 nodes and 78 edges in the exogenous community; Figure 3b and c) and the same proportion of negative partial correlations (respectively 19.0 and 19.4% in N and E). The network constructed from the data stemming from the sterilised soil included a higher number of nodes and edges than the other networks (100 nodes, 176 edges; Figure 3a) and a smaller proportion of negative partial correlations (2.3%). Note that the number of nodes and edges in the networks is not related to the community richness (Karimi et al. 2017) and that the networks draw a picture of an emerging pattern at the microbial context scale rather than the pot scale (at which  $\alpha$ -diversity indices are calculated). The three networks showed high proportions of specific nodes: 72, 51 and 44% in network S, N and E respectively (calculated as the proportion of specific nodes over the total number of nodes in a given network; Figure 3d).

### *Soil microbial context effect on plant biomass and soil nitrogen content*

Our results showed that the microbial context significantly influenced plant biomass (Table S5, Figure 4). Overall, shoot biomass was increased by 10.6% and 5.5% in the exogenous (E) and native (N) microbial contexts respectively as compared to the sterilised soil (S; Figure 4). There was an interaction between the soil microbial context and the plant diversity ( $p_{df=14} = 4.9 \times 10^{-3}$ ; Table S5). Indeed, wheat species monocultures (W1 and W3) tended to respond differently than the other plant diversity treatments to the soil microbial context (Figure 4a). The lowest wheat shoot biomass occurred with the native microbial community while biomass tended to be higher and similar in S and E (Figure 4a). The overall effect on root biomass was the opposite of that on shoot biomass: we observed a higher root biomass in S ( $0.35 \pm 0.07$  g) than in E ( $0.30 \pm 0.06$  g) with an intermediate value in N ( $0.33 \pm 0.05$  g; Figure 4b). Thus, the microbial context influenced the shoot-root ratio ( $p_{df=2} = 3.3 \times 10^{-9}$ ; Table S5; Figure 4c), i.e. plant resource allocation, but not the total biomass production (shoots + roots;  $p_{df=2} = 0.36$ ; Table S5). To go beyond the three microbial contexts, we noticed that the soil bacterial community richness influenced positively plant total biomass ( $p_{df=1} = 0.048$ ; slope =  $1.89 \times 10^{-4}$ ; adjusted  $R^2 = 0.17$ ; Figure S3). It suggested a possible influence of the bacterial community diversity per se on plant productivity, which requires further dedicated investigations.

Total soil mineral nitrogen depended on the microbial context ( $p_{df=2} = 2.4 \times 10^{-7}$ ; Table S6) and on the plant diversity (i.e. the eight plant diversity treatments + control;  $p_{df=8} < 2.2 \times 10^{-16}$ ; Table S6). It was reduced in S and N as compared to E (respectively  $5.2 \pm 9.7$ ,  $6.3 \pm 8.9$  and  $10.3 \pm 10.3$  mg.kg<sup>-1</sup> dry soil on average; Figure S4). Soil mineral nitrogen content was also reduced in pots containing plants as compared to control pots (respectively  $4.3 \pm 5.5$  and  $29.7 \pm 6.3$  mg.kg<sup>-1</sup> dry soil on average, of which respectively 91.3 and 85.6% were ammonium; Figure S4). Interestingly, pea species monocultures (i.e. P1 and P3) tended to show a higher soil mineral nitrogen content than the other treatments involving plants. This effect seemed to be driven by high nitrogen content in the presence of the exogenous microbial community (Figure S4;  $p_{df=16} = 0.065$  for the interaction between plant diversity and microbial context; Table S6).

### *Interactions between plant diversity effects and soil microbes*

The microbial context did not only affect plant productivity but also the existence of plant diversity effects on plant biomass. Indeed, there was no significant effect of increasing either species or genotypic diversity on shoot biomass in the sterilised soil, but positive effects in microbial contexts N and E (Table 1). In the native microbial community, we also registered a negative effect of increasing pea genotypic diversity (Table 1). The effect of mixing both diversity levels on shoot biomass (i.e. change in biomass in W1P3, W3P1 and W3P3 as compared with W1) was never significant in S, significant in W3P1 in E and both in W3P1 and W1P3 in N (Table 1).

The shoot biomass deviation from the additivity hypothesis, i.e. the existence of an interaction effect between species and genotypic diversity, depended on the microbial context ( $p_{df=2} = 9.4 \times 10^{-5}$ ; Table S2). There was a positive deviation from additivity in N and E ( $+0.13 \pm 0.09$  g and  $+0.08 \pm 0.12$  g respectively i.e. +15.8 and + 8.9%) whereas there was no effect in the sterilised soil ( $-0.03 \pm 0.09$  g i.e. -3.7% which was not significantly different from zero; Figure 4d). Interestingly, whereas the highest shoot biomass occurred with the exogenous community (Figure 4a), the highest deviation from additivity tended to be achieved with the native community (Figure 4d).

An assessment of shoot biomass per capita allowed investigating potential mechanisms underlying these deviations from additivity. We showed that pea shoot biomass was not affected by the association with wheat ( $p_{df=1} = 0.91$ ) whereas wheat shoot biomass increased when associated with pea ( $p_{df=1} = 4.4 \times 10^{-9}$ ) which seemed to depend on the microbial context ( $p_{df=2} = 0.06$  for the interaction term; Table S7). More particularly, the shoot biomass of the genotype RE13088 increased in mixture as compared to monoculture only in N and E (Figure 5). It led to a reduction in the productivity differential between the three wheat genotypes (standard deviations in Figure 5). However, this was not confirmed by the three-way interaction genotype\*microbial context\*species diversity ( $p_{df=4} = 0.35$ ; Table S7).

#### *Plant effect on soil bacterial communities*

The soil bacterial OTU richness in pots with plants was similar to that of the control without plant ( $1208 \pm 316$  and  $1195 \pm 340$  OTUs respectively). The OTU richness in the inoculated soils at the end of the experiment was lower than the OTU richness in the initial inoculums (i.e. the liquids used for soil inoculation;  $2023 \pm 333$  and  $2288 \pm 104$  OTUs in inoculums N and E respectively) but again, it did not differ between pots with plants and control pots (respectively  $-743 \pm 134$  and  $-744 \pm 141$  OTUs as compared to the mean initial richness). Moreover, there was no difference in the bacterial community structure either considering the detail of the plant diversity treatments (the eight plant diversity treatments + control) or the plant species treatments (wheat, pea, mixture, control). We detected significant effects of the plants on soil bacterial communities performing PERMANOVA but these effects were weak ( $R^2 = 0.06$  and  $0.03$ ; Table S3 and Table S8) and not detected by pairwise comparisons after correction for multiple testing. However, we noticed that there was a stronger divergence of the bacterial community structure across treatments in the sterilised soil than in the two other microbial contexts (Figure 6; see Figure S5 for a detailed view of each microbial context).

## **Discussion**

The purpose of this study was to assess the effect of soil microbes on the interaction between plant genotypic and species diversity. We found that the influence of the soil microbial context on plant productivity occurred in three ways: i) through effects on shoot and root biomass (and thus differences in shoot-root ratio; Figure 4c), ii) by regulating the significance of species and genotypic diversity

effects on shoot biomass (Table 1) and iii) by modulating the effect of the interaction between species and genotypic diversity on shoot biomass (Figure 4d).

Soil sterilisation and inoculation or not with a soil suspension successfully created three different microbial contexts in terms of bacterial community diversity and structure, as attested by the bacterial community diversity metrics and suggested by the network analysis. We found fewer nodes and edges in the networks of inoculated soils than in the network of sterilised soil, i.e. less OTUs that were involved in direct interactions (Chiquet et al. 2021). As the environment was controlled and plant diversity had no effect on soil bacterial communities, it is likely that the differences in network structures reflect the effect of soil inoculation at the beginning of the experiment. We propose that the higher node and edge number in network S could suggest less constraints in soil colonisation dynamics (which could be due to the absence of species with high competitive abilities at the beginning of the experiment due to the absence of inoculation). These differences in soil microbial context determined plant shoot/root ratio as biomass production was more root-oriented in the sterilised soil than in inoculated soils. A substantial difference between the microbial contexts occurred through the establishment of a *Rhizobium*-legume symbiosis in inoculated soil only (N and E). We can hypothesize that it played a role in the observed differences in plant biomass between the microbial contexts, both in pea monocultures and pea-wheat mixtures. Beyond the possible role of mutualist interactions and despite the fact that our experiment was not specifically designed to assess the effect of microbial community richness on plant productivity, we noticed that the relationship between plant total biomass and bacterial community richness tended to be positive within each microbial context (Figure S3), giving rise to a positive overall relationship. Another study, that experimentally manipulated arbuscular mycorrhizal fungi diversity, showed that the fungal community richness affected plant biomass production (either positively or negatively depending on the plant species; Wagg et al. 2011a). More recently, a study highlighted that pea yield (grain biomass per plant) was influenced by the microbial community richness both in a wild-type genotype and a non-nodulating non-mycorrhizing mutant under drought stress (Prudent et al. 2020). Taken together, these results suggest that soil microbial richness per se could be of importance for plant productivity. This effect of richness could occur through sampling effects (e.g. increase in the probability of the presence of a beneficial microbial partner) or involve complex microbe-microbe interactions (e.g. higher availability of a nutrient allowed by the joint presence of several microbial species).

We highlighted that the existence of an effect of either plant species or genotypic diversity on shoot biomass was determined by the soil microbial context. In particular, shoot biomass did not respond to plant diversity in the sterilised soil contrary to what happened in inoculated soils where species diversity had a positive effect on shoot biomass (Table 1). This is consistent with the results of a previous study which showed no effect of the species richness (from one to three grass-land species) or evenness on shoot biomass per plant in a sterilised soil, whereas there was a positive diversity-productivity relationship when the soil was inoculated with a soil microbial community (Wang et al. 2019). In this

study, the authors also included a treatment in which the soil was inoculated with a combination of arbuscular mycorrhizal fungi isolates and they showed no plant diversity effect on productivity, as observed here for the sterilised soil. These results suggest that the plant diversity-productivity relationship is more likely influenced by a complex soil microbial community (e.g. stemming from field soil) rather than a simplified one (e.g. built in the laboratory). By contrast, other studies integrating higher levels of plant species richness (up to eight tree seedling species (Liang et al. 2019) and up to 15 herbaceous plant species (Schnitzer et al. 2011) registered an increase in plant total biomass with species richness increase even in sterilised soils. But, in Schnitzer et al. (2011), the shape of the diversity-productivity relationship was still affected by soil sterilisation. In the sterilised soil, productivity increased linearly with species diversity whereas, in inoculated soils, the diversity-productivity relationship was saturating. The authors suggested that, in the sterilised soil, the positive plant diversity-productivity relationship was mainly driven by plant complementarity whereas, in field soil, microbes had a major role and drove the relationship by strongly reducing plant productivity at low diversity levels (in which disease incidence was higher) giving rise to a non-linear relationship. Together, these results suggest that the diversity-productivity relationship could be more influenced by soil microbes in plant communities with low diversity levels or including species involved in strong mutualistic interactions (e.g. legumes).

To go further, our study provided evidence that the effect of the interaction between species and genotypic plant diversity on productivity can be modulated by the soil microbial community. Indeed, we did not detect plant species and genotypic diversity interaction in the sterilised soil whereas, in inoculated soils, plant productivity in species and genotypic mixtures was higher than expected in the absence of interaction. We expected the interaction effect on productivity to be different between the two inoculated soils but the gain of shoot biomass due to species and genotypic diversity interaction was similar. In both inoculated soils, the interaction effect seemed to be partly driven by changes in the effect of wheat genotypic diversity in pea/wheat mixtures as compared to wheat monoculture. While wheat genotype performances changed when associated with pea in inoculated soils, the relative performances remained similar to those observed in wheat monoculture in the sterilised soil. In the light of the knowledge on pea/wheat mixture (Bedoussac and Justes 2010; Bedoussac et al. 2015; Duchene et al. 2017), we suggest that the presence of nodules on pea roots in inoculated soils allowed niche complementarity to occur in pea/wheat mixtures thus reducing competition, and more specifically intraspecific competition (Mahaut et al. 2020), for nitrogen. This interpretation is supported by very low levels of mineral nitrogen in the sterilised soil as compared to inoculated soils. Previous studies showed that complementarity allowed a better resource use which could explain a gain in productivity in mixtures as compared to monoculture (Mason et al. 2020), here our results suggest that complementarity in nitrogen use in species mixture could prevent nitrogen-limitation (which is likely to occur in our experiment in pots) and allow positive interactions between genotypic and species diversity.

Rather than a synergy between two positive effects of species and genotypic diversity, we observed that species mixture compensated the negative effect of genotypic diversity. Previous studies highlighting interactions showed that increasing genotypic diversity in a species mixture could change the effect of species diversity on above-ground productivity, either in a positive (switch from a negative to a positive relationship; Crawford and Rudgers 2012) or a negative way (decrease in net biodiversity effect; Schöb et al. 2015). The underlying mechanisms imply changes in interspecific competition and in species dominance patterns (Fridley and Grime 2010; Schöb et al. 2015). Here, we provided a first evidence that changes in dominance patterns could also be involved in the species diversity effect on the genotypic diversity-productivity relationship, and that, these mechanisms can be driven by the soil microbial communities.

A classical objective of diversity-productivity research is to shed light on genotypic and species interactions among plants. Some studies are interested in including plant interactions with other organisms, such as microbes, as modulators of this relationship. Based on our results and in the light of the concept of holobiont, it would be particularly interesting to assess the relative weight of inter-kingdom (plant-microbes) interactions as compared to intra-kingdom (plant-plant) interactions in explaining the biodiversity-ecosystem functioning relationship.

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### **Code availability**

The scripts used during the current study are available from the corresponding author.

### **Authors' contributions**

Manuel Blouin conceived the study. Tiffany Raynaud performed the experiment. Aym  Spor and Tiffany Raynaud analysed the data. Aym  Spor, Manuel Blouin and Tiffany Raynaud wrote the manuscript with substantial contributions of Barbara Pivato and Mathieu Siol.

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**Data availability**

The datasets generated and/or analysed during the current study are available from the corresponding author. Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-021-05071-z>.

**Conflict of interest**

The authors have no relevant financial or non-financial interests to disclose.





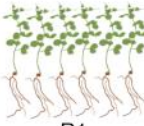





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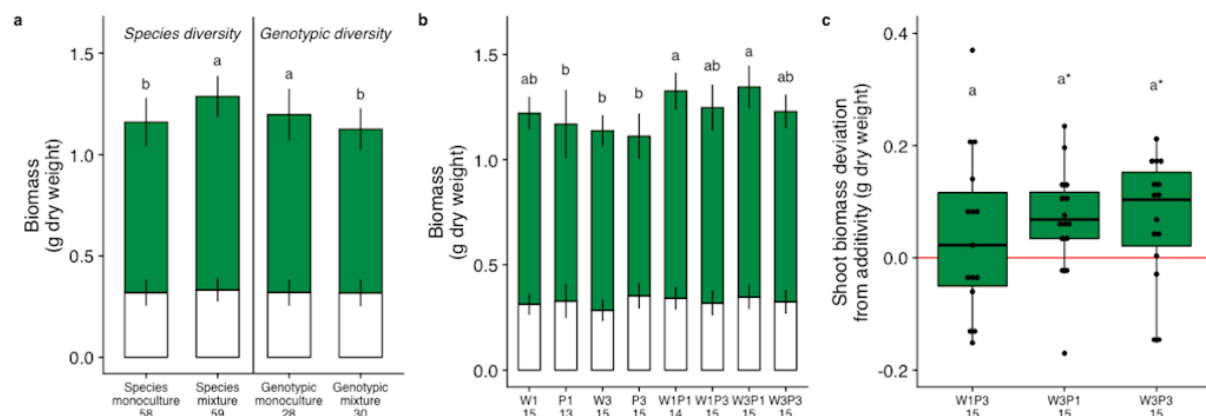
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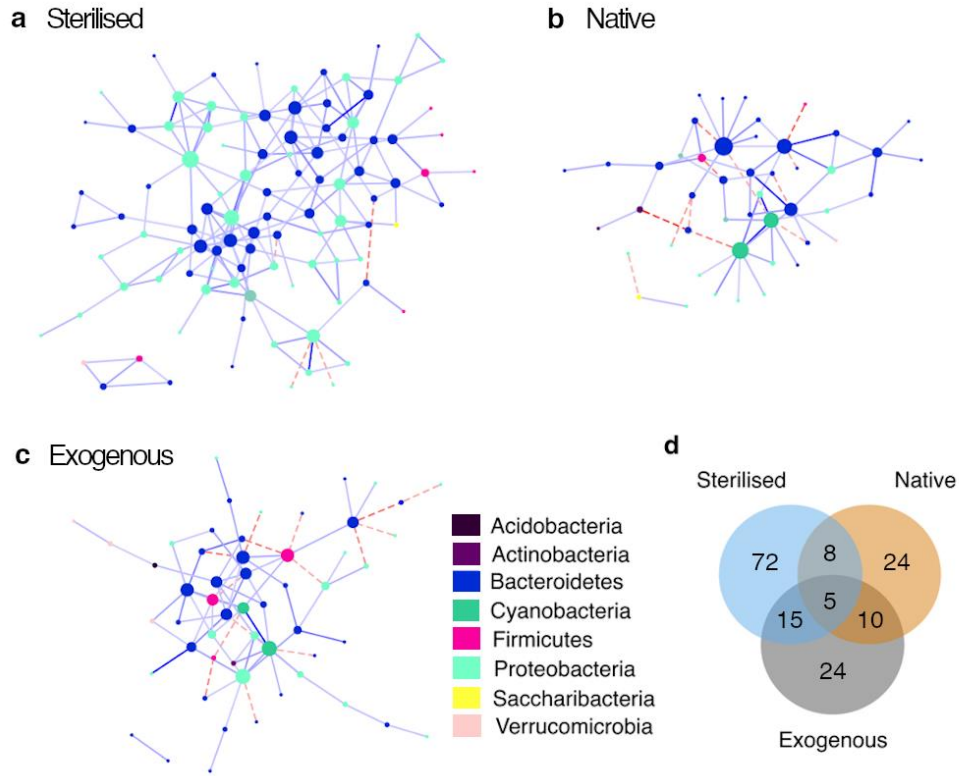
		Number of wheat genotypes		
Number of pea genotypes		0	1	3
	0	Control	 W1	 W3
	1	 P1	 W1P1	 W3P1
	3	 P3	 W1P3	 W3P3

**Figure 1: Experimental design.** Two plant species, pea and wheat, were grown under two levels of species diversity, i.e. monoculture or pea- wheat mixture, and two levels of genotypic diversity, i.e. one or three genotypes per species. Abundance was fixed to six plants per pot. W: wheat; P: pea; 1: one genotype; 3: three genotypes. W1 and P1 refer to the genotypes Flamenko and Cameor respectively and W3 and P3 refer to the association of Flamenko, Renan, RE13088 and Cameor, Champagne, 336.11 respectively

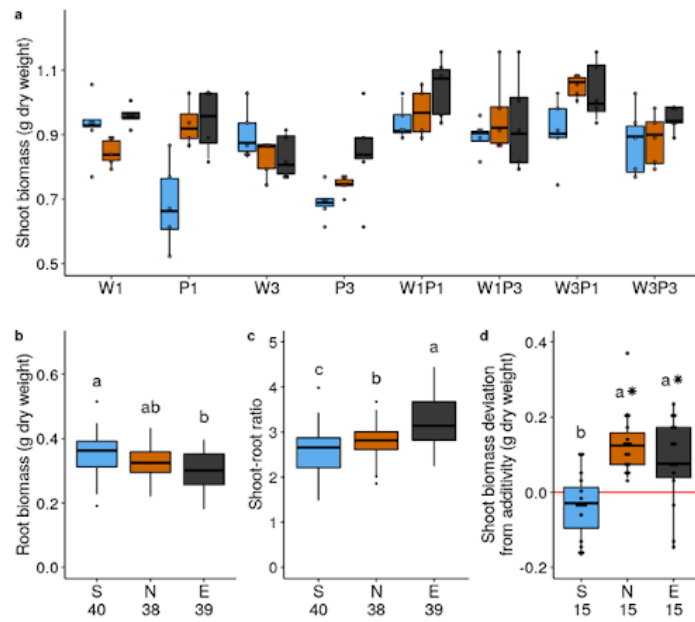


**Figure 2: Plant diversity effect on plant biomass.** Plant biomass at the pot scale according to a. species or genotypic diversity (species monoculture: W1, P1, W3, P3; species mixture: W1P1, W1P3, W3P1, W3P3; genotypic monoculture: W1, P1; genotypic mixture: W3, P3) and b. plant diversity treatment (W: wheat; P: pea; 1: one genotype; 3: three genotypes). Bottom: root biomass; top: shoot biomass. Letters represent significant differences in total biomass production ( $\alpha = 0.05$ ). Bars represent the standard deviation. c. Shoot biomass deviation from the additivity hypothesis (i.e. the effect of combining species and genotypic diversity on plant biomass is equal to the sum of the effects of species diversity and genotypic diversity) depending on the mixture diversity. Letters represent significant differences between the treatments ( $\alpha = 0.05$ ). Asterisks represent significant differences from zero. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. Sample sizes are given on the bottom of the graphs.



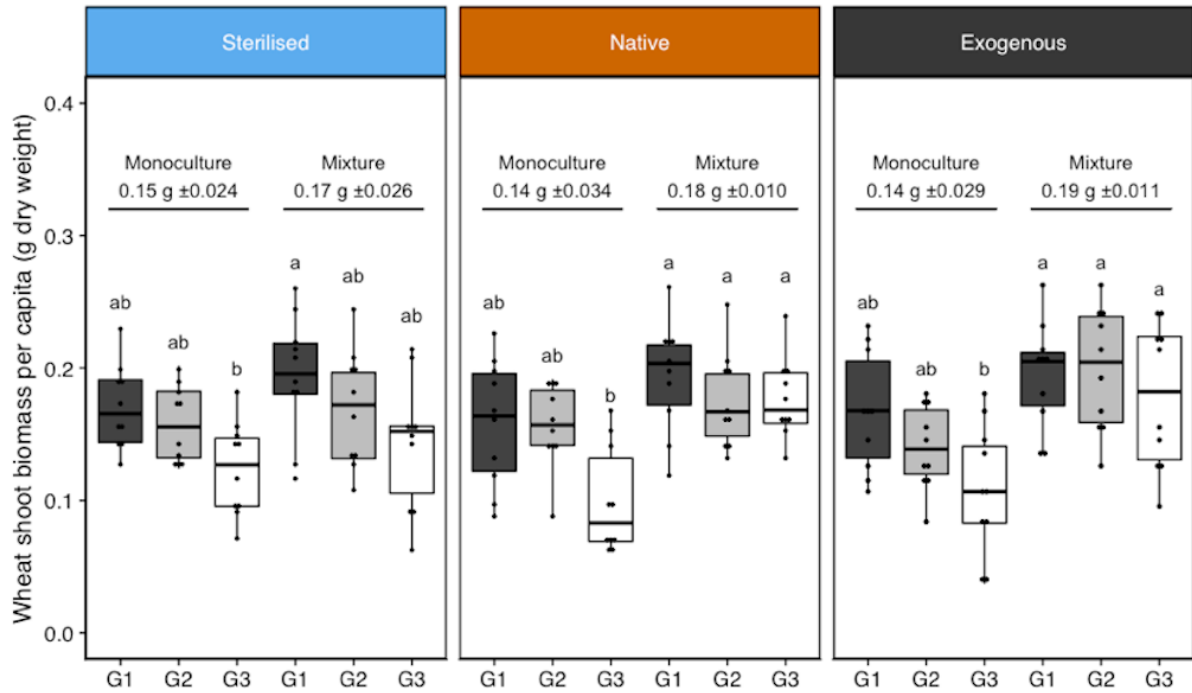


**Figure 3: Correlation networks of the soil bacteria depending on the microbial context.** a. Sterilised b. Native c. Exogenous. Nodes represent OTUs, edges represent partial correlations (threshold = 0.075; dashed line:  $<-0.075$ ; solid line:  $>0.075$ ). Node colour depends on the OTU phylum and node size depends on the number of nodes connected to the considered node. The networks were built based on the partial correlation values given by a Poisson log-normal model selected with StARS method. d. Venn diagram representing the number of specific and shared nodes in the three networks. Only partial correlations superior to 0.075 in absolute value were represented for readability but note that the obtained networks and the corresponding Venn diagram give a good representation of the differences between the three microbial contexts as compared to what is obtained when all partial correlations are considered (i.e. similar relative number of nodes and edges, similar relative proportion of negative partial correlations and similar levels of node specificity).

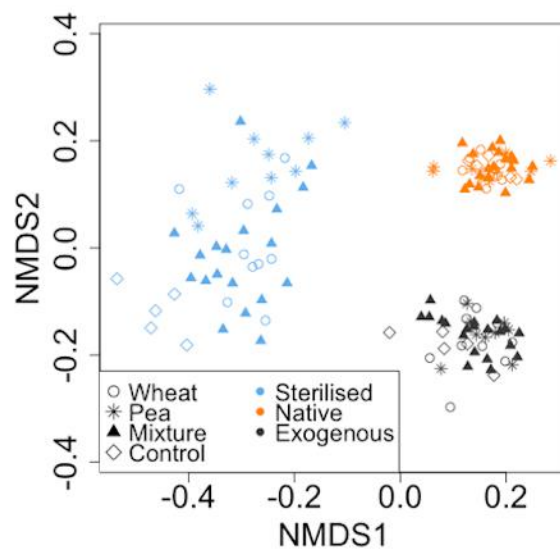


**Figure 4: Plant biomass response at the pot scale to the soil microbial context.** a Shoot biomass depending on the plant diversity, b Root biomass, c Shoot-root ratio. S: sterilised; N: native; E: exogenous. Letters represent significant differences ( $\alpha = 0.05$ ). Letters were not shown on fig. 4a for readability. There was no significant difference within plant diversity treatments except between S and N, E in P1. d. Shoot biomass deviation from the additivity hypothesis (i.e. the effect of combining species and genotypic diversity on plant biomass is equal to the sum of the effects of species diversity and genotypic diversity). Letters represent significant differences between the microbial contexts ( $\alpha = 0.05$ ). Asterisks represent significant differences from zero. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. For panel a, sample sizes are  $n=5$  except for P1 N and E and W1P1 N for which  $n = 4$ . The sample sizes for the other panels are given on the bottom of the graphs.





**Figure 5: Wheat shoot biomass per capita depending on species diversity and soil microbial context.** Monoculture: W3; Mixture: W3P1, W3P3. G1: Flamenko (black); G2: Renan (grey); G3: RE13088 (white). Letters represent significant differences ( $\alpha = 0.05$ ), they are presented for each microbial context independently for readability but were obtained from a post-hoc test following a global model including the three microbial contexts. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers.  $n = 10$ . For each microbial context, the average shoot biomass per capita in monocultures and mixtures is given  $\pm$  inter-genotype standard deviation.



**Figure 6: Non-metric multidimensional scaling of soil bacterial community structure.** Dissimilarities were calculated with Bray-Curtis index. One point per pot. Stress = 0.11

**Table 1: Plant species and genotypic diversity effects on shoot biomass at the pot scale depending on the microbial context.**

		Sterilised	Native	Exogenous
Wheat genotypic diversity		-0.017 g [-0.12;0.091]	-0.017 g [-0.072;0.035]	-0.13 g [-0.18;0.074]
Pea genotypic diversity		+0.0034 g [-0.11;0.12]	-0.19 g [-0.26;-0.13]*	-0.10 g [-0.26;0.044]
Species diversity		+0.015 g [-0.069;0.11]	+0.12 g [0.043;0.20]*	+0.089 g [0.0082;0.17]*
Species and genotypic diversity	W1P3	-0.029 g [-0.12;0.060]	+0.11 g [0.023;0.22]*	-0.021 g [-0.13;0.10]
	W3P1	-0.012 g [-0.13;0.10]	+0.20 g [0.16;0.25]*	+0.080 g [0.0074;0.16]*
	W3P3	-0.041 g [-0.15;0.074]	+0.041 g [-0.027;0.11]	-0.010 g [-0.052;0.031]