

# Increasing diversity gradient in lentil mixtures for intercropping with wheat reveals an increase in lentil biomass with minimal impact on mycorrhizal activity

Elisa Lorenzetti, Federico Leoni, Gilbert Koskey<sup>\*</sup>, Paolo Bàrberi

Scuola Superiore Sant' Anna, Center of Plant Sciences, Group of Agroecology, Piazza Martiri della Libertà, 33, 56127, Pisa, Italy

## ARTICLE INFO

### Keywords:

Agroecology  
Soil health  
Functional biodiversity  
Lentil landraces  
Ancient and modern cultivars

## ABSTRACT

The management of arbuscular mycorrhizal fungi (AMF) is crucial for sustainable agricultural systems, given its beneficial impacts on soil health and crop productivity. This study aimed to assess the effect of lentil (*Lens culinaris* Medik.) mixtures for intercropping with durum wheat (*Triticum durum* Desf.) on mycorrhiza and investigate the role of legume intraspecific diversity in mycorrhizal development and crop biomass production. Since both intraspecific and interspecific diversity (i.e., cultivar mixtures and intercropping) have an effect in shaping soil microbiota in the rhizosphere, we hypothesised that increasing the level of diversification in the system enhances the provision of services (e.g., aboveground biomass accumulation), due to enhanced microbial activity and diversity. Specifically, increasing diversity within lentil cultivar mixtures strengthens the affinity of wheat for mycorrhiza, thereby influencing wheat mycorrhization in wheat-lentil intercrops. To test our hypothesis, we designed three lentil cultivar mixtures with increasing diversity levels using a trait-based approach, drawing from a pool of Italian lentil landraces characterised in a previous study. As an additional factor, we intercropped lentil mixtures with wheat and compared it with sole wheat and sole lentil commercial cultivar. In both experimental years, intercropping lentil mixtures with wheat consistently yielded higher soil mycorrhization levels (46% in 2021 and 52% in 2022) compared to sole wheat cultivation (23% in 2021 and 34% in 2022). Nevertheless, AMF colonisation in lentil roots was significantly higher in sole crops (55% in 2021 and 70% in 2022, except for the commercial cultivar) compared to intercrops (35% in 2021 and 60% in 2022). This difference could be attributed to the presence of wheat, which might have suppressed the capacity of lentils to establish successful symbiosis with mycorrhiza. On the other hand, AMF colonisation of wheat roots in 2022 was significantly higher (52%) when intercropped with lentils than when cultivated as sole wheat (40%). Concerning biomass production in lentil mixtures in 2022, we observed a positive correlation between lentil biomass and the number of functional groups in the mixtures, both as pure stands ( $R^2 = 0.19$ ,  $p = 0.06$ ) and intercrops ( $R^2 = 0.29$ ,  $p = 0.01$ ). Additionally, in 2022, the total biomass of the intercropped system increased with the increase in diversity. Further experiments, incorporating microbial genetic analysis, are necessary to uncover potential associations between functional mixtures and AMF composition.

## 1. Introduction

Ecological approaches in agriculture have demonstrated that higher biodiversity correlates with increased productivity, stability, sustainability, and resilience of the agroecosystems, particularly within organic and low input management systems (Renard and Tilman 2021; Wuest et al., 2021).

Leveraging crop diversity in agriculture may occur at different levels and scales. A first level of genetic diversification may be represented by

cultivar mixtures, i.e., the simultaneous cultivation of multiple cultivars of a given species (Lazzaro et al., 2018). It is widely acknowledged that cultivar mixtures contribute to the reduction of pathogen spread (Newton, 2016), enhancement of production stability, especially under stressful conditions (Creissen et al., 2016; Lorenzetti et al., 2022), and provision of ecological services such as weed control, promotion of beneficial insects, soil quality improvement, and reduction of nutrient leaching (Wuest et al., 2021).

A second level of spatial crop diversification can be achieved through

<sup>\*</sup> Corresponding author.

E-mail address: [gilbert.koskey@santannapisa.it](mailto:gilbert.koskey@santannapisa.it) (G. Koskey).

intercropping, i.e., the cultivation of two or more species in the same field (Vandermeer, 1992). Intercropping is associated with yield gains; for instance, Renard and Tilman (2021) reported a yield surplus in intercrops ranging from +16% to +30% compared to yields from monocultures. Intercropping can contribute to the reduction of synthetic fertilizer use (Jensen et al., 2020), pesticides, and herbicides by improving pest and disease control (Lopes et al., 2016) as well as weed suppression (Leoni et al., 2022; Amossé et al., 2013). The practice is also reported to stabilize yields, diversify farmers' incomes, and promote human health through dietary diversity (Koskey et al., 2022a; Renard and Tilman 2021).

Most of the benefits delivered from cultivar mixtures and intercropping stem from the exploitation of ecological complementarity among crops: each different species/cultivar has a different and complementary contribution to a given ecosystem function (Bärberi and Moonen 2020). Consequently, the total value of ecosystem services provided by crop diversification increases with greater differences and complementarity among crop species/cultivars. Therefore, when utilizing agrobiodiversity to achieve multiple benefits, considerations should be given not only to genetic diversity but also to functional biodiversity (Ranaldo et al., 2020; Moonen and Bärberi 2008). In this context, we adopt the definition of "functional biodiversity" from Moonen and Bärberi (2008) as "that part of the total biodiversity composed of clusters of elements (at the gene, species or habitat level) providing the same (agro)ecosystem service, that is driven by within-cluster diversity". For example, cereal-legume intercropping is considered highly effective due to the combined weed suppression capacity of cereals and enhanced nutrient availability from legume symbiosis with rhizobacteria and mycorrhiza (Bedoussac et al., 2015; Koskey et al., 2023; Layek et al., 2018).

Different species/cultivars may not only complement each other but also enhance each other's functions. In cropping systems, it has been demonstrated that in a fava bean-maize intercropping, maize promoted nitrogen (N) fixation in fava beans by regulating gene expression and root exudates, supported flavonoid synthesis, and increased nodulation (Li et al., 2016). Intercropping effect on root exudates was later supported by several researchers (Liu et al., 2017; Leoni et al., 2021; Chamkhi et al., 2022). As well as different crop species, also different cultivars of the same species may have partially different microbiota. Therefore, increasing diversity through the use of cultivar mixtures in intercropping systems can enrich the total pool of microorganisms in the soil and enhance the provision of below-ground ecosystem services (Mauger et al., 2021; Yang et al., 2019; Xiao et al., 2023). In this study we designed a pot experiment to investigate the effect of intercropping between wheat and lentil cultivar mixtures on soil biological fertility, with a special focus on mycorrhizal inoculum potential (MIP), arbuscular mycorrhizal fungi (AMF) root colonisation, and crop biomass production. The lentil cultivar mixtures were organized into three levels of increasing diversity using a trait-based approach. We hypothesised that increasing the level of diversification in the system enhances the provision of services (e.g., biomass production), due to enhanced microbial activity and diversity. Specifically, increasing diversity within lentil cultivar mixtures reinforces wheat's affinity for mycorrhiza, thereby influencing wheat mycorrhization in intercrops.

The choice to examine mycorrhiza within the soil microbiota was driven by its crucial contributions to nutrient uptake, protection from salinity and drought, and the reduction of toxicity from phytotoxic elements (Ganugi et al., 2019). In the case of legumes, mycorrhiza also enhances N fixation (de Novais et al., 2020). Although cereals generally exhibit less affinity for mycorrhiza compared to legumes, intercropping has been shown to enhance the mycorrhization status of both crops (Harinikumar et al., 1990) or in some cases elevates the mycorrhization levels of the less mycotrophic crop (Koskey et al., 2023). The MIP bioassay provides an estimate of mycorrhizal propagules available in the soil that can actively colonize the host plant (Njeru et al., 2014). In addition, assessing the proportion of roots colonized by mycorrhiza can

suggestively indicate the extent of fungal development in the plant and its potential effect on improving nutritional status and growth (Inggraffia et al., 2019; Koskey et al., 2023). We hypothesize that increasing the diversity in lentil cultivar mixtures may increase affinity for mycorrhiza and, consequently, influence wheat mycorrhization in the intercrop.

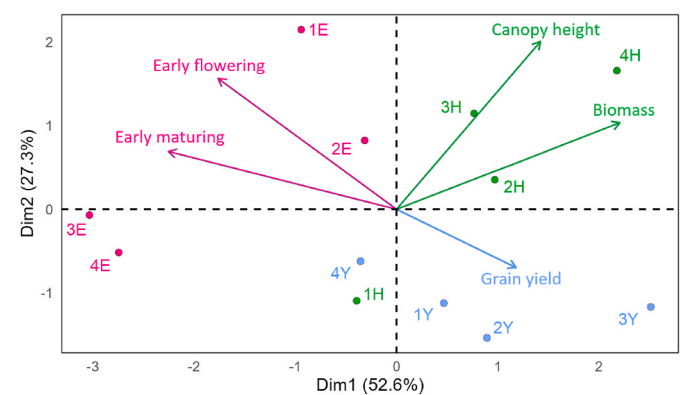
Furthermore, we examined plant biomass to assess the overall growth and vigour of the crops, assuming that higher functional diversity in lentil cultivar mixtures corresponds to increased responsiveness in plant growth and competitive ability with the companion crop.

## 2. Materials and methods

### 2.1. Mixtures compositions

The choice of the lentil accessions was based on the results of a previous characterisation involving a collection of 112 landraces, sourced from seed saver associations and gene banks (Lorenzetti et al., 2023). The agronomic performance and phenotypic characterization of lentils (*Lens culinaris* Medik.) were monitored for two consecutive years in a field adjacent to our pot experiment. Building on an extensive literature review and the findings of Lorenzetti et al. (2023), three target functional traits were selected for the composition of lentil mixtures: (i) early flowering and maturing, aiming for a synchronized harvest time with wheat to benefit from symbiosis before the drought season; (ii) plant height and biomass, for good competitive ability and a wide root surface for microbial interaction (Bektas et al., 2016; Figueroa-Bustos et al., 2018); (iii) grain yield, ensuring a positive land equivalent ratio and incrementing nutrients in the system, as highly productive cultivars are associated with higher nitrogen uptake (Dobert et al., 1993; Roy et al., 2019). For each trait we identified four candidate accessions, thus composing a functional group. The main criterion for accession selection was exclusivity, so that accessions complying with more than one of the chosen traits were discarded (a Principal Component Analysis was performed to observe lentil differentiation based on the target traits, see Fig. 1). In the early group, we included two Italian accessions and two from Ethiopia, whereas for the biomass and yield groups, we used accessions from southern Italy (Table 1).

Mixtures were designed to create a gradient of diversity in terms of the number of species and functional groups. To define the first level of diversity, the early functional group was considered as a baseline (mix 1,



**Fig. 1.** Principal Component Analysis of phenotypic diversity of the lentil cultivars composing the mixtures, based on Lorenzetti et al. (2023) data. Vectors represent the explanatory magnitude and direction of selected traits. Points represent lentil cultivars: Earliness groups is indicated with pink colour and letter E, Height and Biomass group is indicated with green colour and letter H, Yield group is indicated with blue colour and letter Y. Number order within each group represents landrace order in Table 1: 1E from Italy, 2E from Latium's Islands (IT), 3E and 4E from Ethiopia; 1H–4H from Sicily (IT); 1Y from Sicily (IT), 2Y from Abruzzo (IT), 3Y from Basilicata (IT) and 4Y from Campania (IT). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**

Composition of the lentil cultivar mixtures: functional groups and collection site of the components.

Mixture	Functional Groups	Cultivar
Mix 1	Earliness	ITALY, ITALY -Latium's island LT, ETHIOPIA, ETHIOPIA
Mix 1.2	Earliness	ITALY, ITALY -Latium's island LT, ETHIOPIA, ETHIOPIA
	+	+
Mix 1.2.3	Height and Biomass	ITALY -Sicily (Villalba CL, Mussomeli CL, Modica RG, Montagnola PA)
	Earliness	ITALY, ITALY -Latium's island LT, ETHIOPIA, ETHIOPIA
	+	+
	Height and Biomass +	ITALY -Sicily (Villalba CL, Mussomeli CL, Modica RG, Montagnola PA)
	Yield	+
		ITALY -Sicily CL, ITALY -Abruzzo AQ, ITALY -Basilicata PZ, ITALY -Campania CE

with 4 individuals), then the other groups were added incrementally: the biomass group, constituting the mix 1.2 (with 8 individuals), and the yield group (mix 1.2.3, with 12 individuals). To constitute the mixtures, we used a partial replacement approach, reducing the recommended sowing rate of each cultivar to 25% in mix 1, to 12.5% in mix 1.2, and to 8.3% in mix 1.2.3 as compared to pure stands (considering a sowing density of 300 plants m<sup>-2</sup>).

## 2.2. Experimental design

A pot experiment was conducted for two consecutive growing seasons (2021 and 2022) at the Centre of Agro-Environmental Research E. Avanzi (CiRAA) (43.6628N, 10.3485E), 9 km southwest of Pisa, Central Italy. Given the scarcity of literature and empirical data on the use of mixtures in intercropping systems (Kabululu et al., 2014), this preliminary experiment in pots was designed to explore the potentiality of this system in a controlled environment. This approach allowed us to focus on specific interactions between lentil cultivar mixtures and wheat without the interference of weeds, soil variability, and water stress, which can collectively affect the activity of AMF. Additionally, lentil landrace seeds were not readily available, and the quantity at hand was limited.

Durum wheat (*Triticum durum* Desf. cv. Minosse) and lentil mixtures were sown on February 9 and December 16, respectively, for the first (2021) and second (2022) repetitions of the experiment in pots of 27 litres (33 cm x 33 cm x 25 cm). The trial was arranged in a completely randomised block design with 6 replicates and 7 treatments in 2021, and 8 treatments in 2022. The first treatment (mix 1) consisted of a mixture of the early functional group, the second treatment (mix 1.2) included the addition of the biomass group to the early one, and the third treatment (mix 1.2.3) involved the addition of the yield group to the former two. The mixtures were cultivated in intercropping with wheat and as a sole crop. Sole cropping of wheat was sown as a control treatment in both years. During the second repetition of the experiment, we included an additional control treatment of the sole commercial lentil (cv. *Robin*), a Canadian cultivar (Vandenberg et al., 2002), to compare the performance of a pure stand commercial cultivar with that of landrace mixtures.

In the case of intercropping, lentil mixtures and wheat were sown in rows (3 rows of wheat and 2 rows of lentil) with a wheat-lentil row distance of 6 cm. For sole cropping, plants were arranged in 3 or 2 rows, respectively, for wheat and lentils. Plant density was determined according to the standard seeding dose used by farmers in Pisa's plain area. Therefore, lentil mixtures were sown at 300 plants m<sup>-2</sup>, and wheat were sown at the standard dose of 350 plants m<sup>-2</sup>. Due to the high competitiveness of wheat during the first repetition, which resulted in very low lentil biomass, we reduced the wheat density to 116 plants m<sup>-2</sup> during the second repetition (1/3 of the initial dose).

## 2.3. Soil and growing conditions

The experimental site is located in a Mediterranean zone characterized by mild, relatively rainy winters and hot, dry summers.

During the first growing season, the total precipitation in the study period (February–June) was 167 mm, but unevenly distributed, with an atypical deficiency in March (Fig. S1 in Supplementary Material). Additionally, two late frosts occurred in mid-April with temperatures reaching -5 °C before the reappearance of precipitation on the 7<sup>th</sup> and 8<sup>th</sup> of April 2021. The second growing season (December–May) had a total precipitation of 290 mm (Fig. S1 in Supplementary Material). In both years, emergency irrigation was evenly provided to all the pots when necessary.

The plant growth medium consisted of a loamy-sand soil (USDA) comprising 79% sand, 13% loam and 8% clay; organic carbon was 11.16 g kg<sup>-1</sup>, total N 0.8 g kg<sup>-1</sup>, available P 33 mg kg<sup>-1</sup>, total CaCO<sub>3</sub> 21 g kg<sup>-1</sup>, and pH 7.55. The soil type was chosen to enhance drainage and aeration, preventing compaction in the pots and allowing for better root penetration and development. The experiment was conducted according to a low-input management where no fertilisers and pesticides were used, mimicking a nutrient limitation condition to boost microbial symbiosis (Hoeksema et al., 2010).

## 2.4. Sampling and analysis

Crop establishment was assessed on the whole pot surface around 30 days after sowing. Then, at the grain-filling stage (wheat BBCH scale 73, "early milk"), plants were collected: first, the shoots were cut off at ground level and separated by species, dried at 40 °C to a constant weight to obtain the total dry biomass. Secondly, the roots were washed with running tap water and prepared for staining with acidified trypan blue dye following the procedure described by Phillips and Hayman (1970). The percentage of colonized root length was determined with a dissecting microscope at × 40 magnification using the gridline intersect method described by Giovanetti and Mosse (1980).

Before sowing, soil samples were collected from a depth of 20 cm at three randomly selected points in each pot using a 5-cm diameter soil probe. The three sub-samples were mixed to form one homogenous representative sample for each pot and was used to assess MIP at zero point. At the crop termination stage (grain-filling stage), 200 g of soil was collected from the root rhizosphere of lentils and wheat. In the intercrop pots, the rhizosphere soil from both species was pooled to form a homogenous sample. The soil from each pot was used to assess mycorrhizal activity in the soil following the soil MIP bioassay described by Njeru et al. (2014). Soil MIP is commonly used as a biological indicator of mycorrhizal propagules to colonize plant roots within a given period (Njeru et al., 2014; Koskey et al., 2022b).

## 2.5. Statistical analysis

Statistical analyses were carried out in R environment version 4.1.0 (R Core Team 2021). Depending on the data type and error distribution, linear and generalized linear mixed-effects models were fitted using the R/lme4' package (Bates et al., 2015). Wheat, lentil dry biomass (g m<sup>-2</sup>) and cumulative biomass of intercropping (lentil + wheat) were analysed separately using a generalized linear model (LMER), assuming mixtures typology (three levels: mixture 1, 1.2 and 1.2.3), intercropping (two

levels: presence and absence), year (two levels: first and second repetition), and their interactions as fixed terms, with blocks as a random term. To determine significant differences, Sidak post-hoc test was performed to separate means ( $p < 0.1$ ) using the R/‘emmeans’ package (Lenth, 2020). A mixed effect model was used to determine the relationship between lentil mixtures’ biomass production and functional diversity (i.e., the number of functional groups involved in a mixture) separately for intercrops and sole lentil, with blocks as a random effect. Model selection was based on Akaike selection criteria (Zuur, 2009), and only the most conservative model respecting the residual diagnostics was selected. Model comparison was performed using the R/‘AICcmodavg’ package (Mazerolle, 2020). Soil mycorrhizal potential (MIP) and arbuscular mycorrhizal fungi (AMF) colonisation were analysed using a linear mixed-effect model (LME) when normally distributed, and using a generalized linear mixed model (GLMM) (Gamma distribution and identity link function) when not normally distributed. Mixture typology and intercropping were considered as fixed terms, block as a random term. The interaction between year and the explanatory variables was significant, leading to separate analyses for AMF and MIP in the two years. To determine significant differences, Tukey’s post-hoc test was performed to separate means using the R/‘emmeans’ package (Lenth, 2020).

### 3. Results

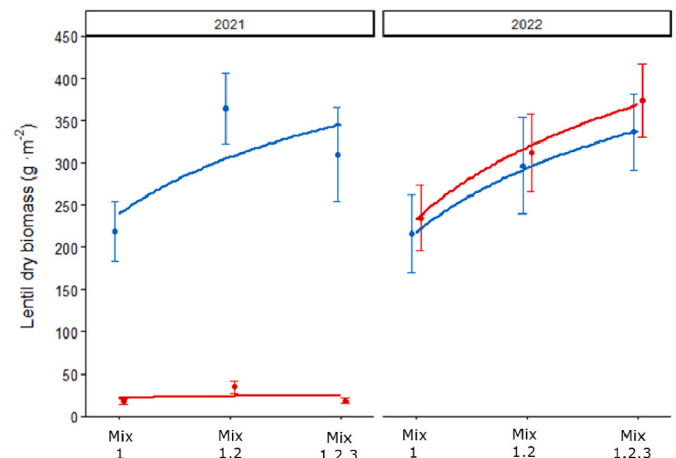
#### 3.1. Biomass production

During the first repetition of the experiment, intercropping with durum wheat negatively affected the lentil biomass due to high competition from the cereal (Fig. 2). Overall, the intercropping with wheat led to a significant reduction in lentil mixture biomass by 92% compared with the biomass of sole lentil mixtures ( $297.5$  vs  $23.5$   $\text{g m}^{-2}$ ). For the sole lentil, the mixture composition significantly affected the biomass production ( $p = 0.008$ ). In particular, biomass production of the lentil mixture 1.2 ( $364.5$   $\text{g m}^{-2}$ ) was significantly higher than mixture 1 ( $218.3$   $\text{g m}^{-2}$ ). Contrary to lentils, wheat biomass was not affected by intercropping ( $p = 0.81$ ), averaging  $357$   $\text{g m}^{-2}$ .

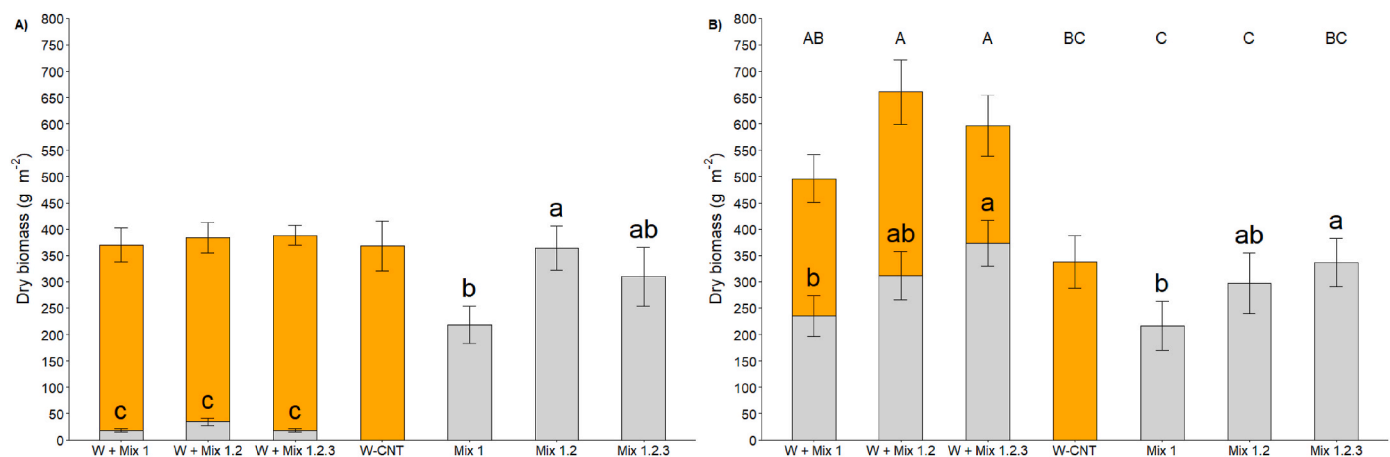
During the second repetition of the experiment, to avoid competition between wheat and lentil observed in 2021, wheat density was reduced by 67% ( $350$  plant  $\text{m}^{-2}$  vs  $116$  plant  $\text{m}^{-2}$ ). In this case, the lentil biomass was significantly affected by mixture composition ( $p < 0.001$ ) but not by intercropping ( $p = 0.39$ ). The biomass production of lentil mixtures was on average  $306$   $\text{g m}^{-2}$  and  $283$   $\text{g m}^{-2}$  for intercropped and sole lentils, respectively. In both intercrops and sole crops, the biomass production

of lentil mixtures 1.2.3 (on average  $355$   $\text{g m}^{-2}$ ) was significantly higher than mixtures 1 (on average  $225.6$   $\text{g m}^{-2}$ ). Wheat biomass was not significantly affected by the lentil mixtures ( $p = 0.18$ ). In the case of intercropping, the combined biomass of wheat and lentils was significantly higher compared to that of the sole lentil mixtures ( $p < 0.001$ ). Specifically, the total biomass of mixture 1.2 ( $660$   $\text{g m}^{-2}$ ) and 1.2.3 ( $596$   $\text{g m}^{-2}$ ) was significantly higher than lentil mixtures cultivated as sole crops (on average  $283$   $\text{g m}^{-2}$ ).

In 2022, the relationship between lentil biomass and the number of functional groups in mixtures was slightly significant in sole crops ( $R^2 = 0.19$ ,  $p = 0.06$ ) and significant in case of intercropping with wheat ( $R^2 = 0.29$ ,  $p = 0.01$ ) (Fig. 3). In 2021, lentil mixtures as sole crop showed a similar trend, but not significant ( $R^2 = 0.16$ ,  $p = 0.13$ ), while no trend was detected for lentil mixtures in intercropping.



**Fig. 3.** Relationship between the number of functional groups in lentil mixtures and biomass production as observed in intercrops with wheat (red lines) and as a sole crop (blue line). In 2022, a significant correlation was found in intercropping ( $R^2 = 0.29$ ,  $p = 0.01$ ), while a marginal significance was observed in sole crops ( $R^2 = 0.19$ ,  $p = 0.06$ ). In 2021, lentil mixtures as sole crop showed a similar trend, but not significant ( $R^2 = 0.16$ ,  $p = 0.13$ ), while no trend was detected for lentil mixtures in intercropping. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 2.** Dry biomass production of lentil mixtures (grey) and wheat (orange) as pure crop or intercrop in 2021 (A) and 2022 (B). Different lowercase letters indicate statistically significant differences ( $p < 0.1$ ) for lentil biomass (Sidak post-hoc test). Different uppercase letters indicate statistically significant differences ( $p < 0.1$ ) for the total biomass (Sidak post-hoc test). Error bars represent the standard errors of the means (SE). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



### 3.2. Mycorrhizal inoculum potential in the soil

In both experimental years, the mycorrhizal inoculum potential (MIP) in the soil at sowing time did not exhibit significant differences between treatments, with a cumulative average of 17% in 2021 and 19% in 2022.

In 2021 and 2022 cropping seasons, the MIP at harvest did not show significant differences among treatments containing lentils, whether as mixtures (intercrops with wheat or sole crops), or as the cv. *Robin* in 2022. However, soil MIP was notably lower in the pots with sole wheat (Fig. 4).

### 3.3. Arbuscular mycorrhizal fungi in the roots

In the 2021 cropping season, the colonisation levels in lentil roots did not show significant differences among the mixture levels but showed only among the intercropping levels. Lentils grown in intercrop conditions showed reduced root colonisation levels (cumulative average of 35%) compared to that of the sole lentils (cumulative average of 55%). Regardless of the mixture level of the companion crop or the intercropping level, AMF root colonisation in wheat remained consistent, averaging approximately 14% (Fig. 5).

In the 2022 cropping season, the trend remained the same as the previous year, but with an increase in the percentage of colonisation: ca. 60% in intercropped lentils, and ca. 70% in the lentils grown alone. Conversely, cv. *Robin*, despite being cropped as a pure stand, had a significantly different colonisation level from the mixtures grown alone, showing lower AMF root colonisation (60%). Concerning AMF colonisation in wheat, a significant difference emerged between wheat intercropped with lentil mixtures (ca. 52% of root colonisation) and that of the pure wheat stands (ca. 40%) (Fig. 5).

## 4. Discussions

### 4.1. Biomass

#### 4.1.1. Wheat and total biomass

In accordance with the findings of the previous studies (Koskey et al., 2022b; Geren et al., 2008), the biomass of both lentils and wheat in 2022 was generally unaffected by intercropping, except for mix 1.2.3 where wheat biomass experienced a reduction, though without impacting the total biomass of the intercrop. The total biomass produced by the intercrop in 2022 was significantly higher than the biomass of the sole

crop. At the level of spatial diversification, the 2022 results confirm the diversity-productivity hypothesis (Tilman 2001), asserting that the higher the diversity in a limiting environment, the higher the total biomass.

The yearly difference in biomass production of intercropped lentils is probably due to two main factors: i) in 2021, the cropping season commenced with harsh climatic conditions, characterized by low precipitation and late frosts, which slowed down lentil development. ii) Wheat biomass in 2021 was three times higher than in 2022 (full dose vs 1/3 dose), imposing strong competition on lentils and impeding recovery when climatic conditions became more favourable. Lentils grown alone could fully develop from mid-April, even though the biomass response to diversity showed different trends than in 2022.

In 2021, the diversity levels of lentil mixtures did not significantly affect biomass production ( $p = 0.16$ ). The influence seems to be driven by functional groups, with mix 1.2 leading (containing the highest share of biomass functional group at 50%), followed by mix 1.2.3 (25% of the biomass functional group). The harsh climate may have played a role in selecting the most adapted functional group. However, in 2022, trends in lentil biomass support the diversity-productivity hypothesis (Tilman 2001) at the level of cultivar (genetic) diversification: biomass production increased with the increase in functional group diversity in the mixtures, both when grown alone ( $p = 0.01$ ) and in intercrop ( $p = 0.06$ ). A recent study (Schnitzer et al., 2011) suggests that the driver for the diversity-productivity phenomenon may not be the niche-complementarity effect, as originally theorised, but the associated microbial activity and diversity. Niche-complementarity effect argues that increased productivity in diversified systems is due to complete resource utilization thanks to species specialization in resource use (Schnitzer et al., 2011).

### 4.2. Mycorrhizal response to lentil mixtures and intercropping

In both experimental years, the mycorrhizal inoculum potential (MIP) in the soil at sowing time was lower than that at crop harvesting time, indicating an increase in MIP with crop development. This is in line with the previous observations of Turrini et al. (2018) and Njeru et al. (2014), who similarly observed an increase in AMF activity across plant developmental stages. Different crop species have differing affinities to AMF and sometimes, the identity of plant host and fungal symbiont may induce a stronger selection effect than that of a rich or diversified plant-AMF communities (Turrini et al., 2018; Koskey et al., 2023). However, in our study, no differences in MIP were observed

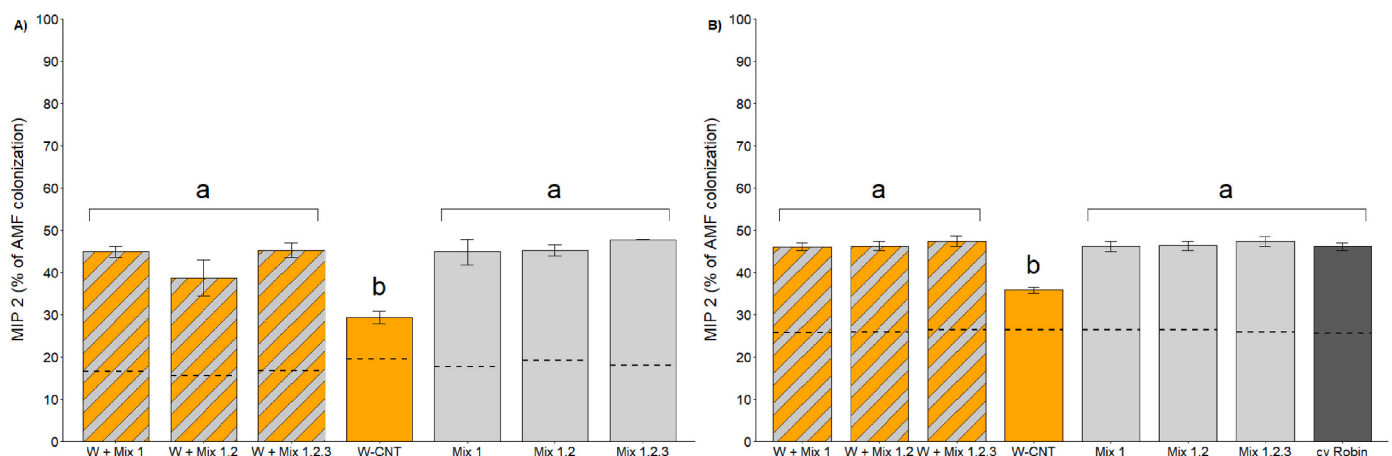
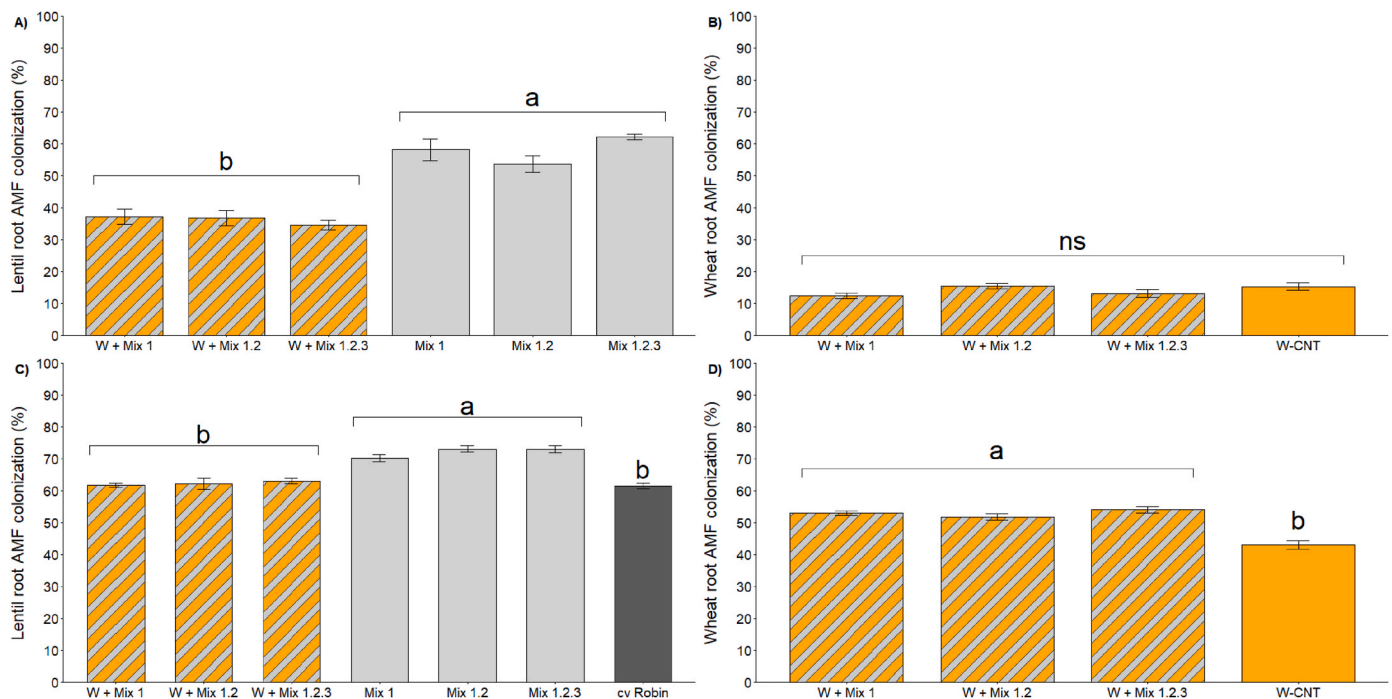


Fig. 4. Soil mycorrhizal inoculum potential (MIP) activity as influenced by intercropping (orange-grey striped bars), cultivar mixtures (grey bars, Mix 1, Mix 1.2, Mix 1.2.3) and sole wheat (orange bar) during 2021 (A) and 2022 (B). In figure B) dark grey for commercial cv. *Robin*. Lowercase letters indicate statistically significant differences between treatments at 0.05 level (Tukey's post hoc test). Error bars represent the standard errors of the means. For each treatment, the bars indicate the mean MIP values at grain-filling stage, the hatching indicates the mean MIP values before sowing. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 5.** Root mycorrhizal colonisation (AMF) in 2021 (A, B) and 2022 (B, C) respectively for lentil (A and C) and wheat (B and D). Grey-orange striped bars represent intercropping (W + Mix 1, W + Mix 1.2, W + Mix 1.2.3), whereas light grey and orange bars refer respectively to sole lentil mixtures (Mix 1, Mix 1.2, Mix 1.2.3) and sole wheat (W-CNT). Dark grey bar represents the commercial lentil cultivar *cv. Robin*. Lowercase letters indicate statistically significant differences between treatments at 0.05 level (Tukey's post hoc test). Error bars represent the standard errors of the means. For each treatment the bars indicate the mean AMF values at grain-filling stage. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

among treatments containing lentil mixtures, whether as intercrops with wheat or sole crops. This suggests that the mycorrhizal activity induced in the soil was the same in all the treatments containing lentil mixtures. As expected, sole wheat induced lower soil mycorrhization in comparison with the lentils, which agrees with the findings of Talukdar and Germida (1994), who observed that wheat is less mycotrophic than lentils.

Nevertheless, AMF colonisation in lentil roots was significantly higher in sole crops (except for *cv. Robin* in 2022) compared to that of intercrops in both years. This contrasts with MIP performances, that did not show any difference between intercropped and sole lentils. MIP is an indicator of mycorrhizal propagules present in the soil and ready to colonize plant roots (Njeru et al., 2014). The soil MIP differences observed between the sole and intercropped lentils could be explained by the presence of wheat, which may have suppressed the capacity of lentils to interact with mycorrhiza (Talukdar and Germida, 1994). This suppression could be attributed to allelopathic compounds released by wheat into the soil, interfering with the signalling between lentil roots and the mycorrhizal fungi in the soil (Javaid 2008). The observed variations in AMF colonisation from year to year could be due to differences in plant development, which were in turn influenced by varying meteorological conditions and competition among the plants. High wheat density, experienced in 2021 intercropped treatments, may have asserted a stronger competition, suppressing lentil growth in the mixtures. Similar competition effect has been reported in another study (Koskey et al., 2022b).

In contrast, AMF colonisation of wheat roots in 2022 was significantly higher when intercropped with lentils than when cultivated as sole wheat, even though, still significantly lower than any lentil treatment. This suggests that the more aggressive and competitive plant, in this case wheat, may have benefited from the facilitation of root-mycorrhiza-dependent biochemicals released by lentils, which may have led to the enhanced root AMF colonisation. This may have implications on nutrient uptake and plant development, especially, under

poor soil conditions. In 2021, due to the very low lentil biomass, intercropped and sole wheat did not show differences in AMF colonisation, but there were significant differences in terms of propagule formation (MIP). This implies that the presence of a legume in the intercrop, even if the crop failed to reach its full growth potential, could have a residual effect in the soil in terms of MIP by favouring the formation of mycorrhizal propagules beneficial for subsequent crops in the rotation (Jie et al., 2013). However, this hypothesis requires validation in a long-term rotational study considering the use of the same field/pot for subsequent years.

Concerning lentil mixtures, we could not detect any effects of diversity levels on MIP and AMF root colonisation. However, we suspect that, rather than affecting AMF quantity, lentil diversity may have influenced AMF and microbiota quality, which was not explored in this study. The increase in lentil biomass may have been driven by the increase in microbiota diversity and quality, as observed in previous studies (Schnitzer et al., 2011; Njeru et al., 2017; Turrini et al., 2018). Therefore, we recommend further research focusing on the genetic characterisation of AMF and rhizosphere microbiota in order to validate this emerging hypothesis. In addition, investigating the long-term effects of interspecific plant diversity on soil microbial composition and diversity and, subsequently, on farming system health, appears to be a promising field for future investigations, considering the services provided in short-term observations (Van Der Heijden et al., 2008; Sessitsch et al., 2018). Interesting results in this direction indeed were observed in grasslands (Eisenhauer et al., 2010).

#### 4.3. Mycorrhizal response to landrace mixtures and commercial cultivar

The lower AMF colonisation in lentil *cv. Robin* compared to that of the landrace mixtures can be elucidated through insights from the studies of Valente et al. (2019) and Mauger et al. (2021). Their findings demonstrated variations in microbial composition between ancient and modern wheat cultivars. Similar results have been reported when

comparing wild, traditional, and modern cultivars of Barley (Bulgarelli et al., 2015) or lettuce cultivars and their ancestors (Cardinale et al., 2015). This discrepancy appears to be related to the capacity of ancient cultivars to actively filter the microorganisms, promoting the beneficial symbiosis first in the rhizosphere through root exudates and then in the root tissues. Modern cultivars may have lost this ability due to breeding efforts focused on increased yield, leading to a loss of genetic diversity at the expense of genes controlling microorganisms filtering (Valente et al., 2019; Mauger et al., 2021). In addition, according to Mauger et al. (2021), this loss has implications in enhancing susceptibility of the breeds to pathogenic microbes.

In addition, the different levels of intraspecific diversity (sole crop vs cultivar mixture) may have played a role. Mauger et al. (2021) demonstrated that cultivar mixtures can have a synergistic effect on the selection of fungal microbiota. It can be hypothesised that the higher AMF colonisation in cultivar mixtures is as a result of increased AMF diversity induced by cultivar diversity (Jie et al., 2013). Alternatively, the lower AMF colonisation in cv. *Robin* may be attributed to competition with other soil microorganisms (Leigh et al., 2011) not adequately filtered (Valente et al., 2019). Even though a recent meta-analysis has excluded the effect of breeding on mycorrhizal affinity (Lehmann et al., 2012), it did not take into consideration the entire soil microbiota, referred to herein as microbial diversity, and the resulting interaction effect on mycorrhizal colonisation. Further experiments are required to confirm this hypothesis: e.g., genetic characterisation of the soil microbiota could deepen the understanding of AMF dynamics and possibly highlight new insights concerning functional mixtures. Moreover, additional research is needed to compare how individual lentil landraces and commercial varieties influence mycorrhizal associations. This will help discern whether the observed effects on mycorrhizal colonisation are attributed to specific cultivar identity or the overall diversity within the cultivar mixtures.

## 5. Conclusions

This study assesses the effect of wheat-lentil intercropping and the role of intraspecific diversity within intercrop on mycorrhiza and biomass development. AMF colonisation in lentil roots was significantly higher in sole crops (except for the commercial control) compared to intercrops. Conversely, AMF colonisation of wheat roots in 2022 was significantly higher when intercropped with lentils. This observation was not confirmed in 2021, probably due to the high wheat density, which exerted strong competition on lentils, suppressing their growth. The lower AMF colonisation in the commercial lentil cultivar may be due to the effect of the different microbial composition in ancient and modern cultivars. However, this hypothesis should be tested in further studies that consider analysis of microbial composition and diversity.

Concerning biomass development, yearly differences were noted, likely influenced by environmental effects and varying wheat sowing rates. In 2022, the total biomass produced by the intercrop was significantly higher than the sole crop biomass, thereby supporting the diversity-productivity hypothesis of Tilman (2001) at the interspecific level (the higher the species diversity, the higher the biomass). In addition, the biomass produced by different lentil mixtures was significantly correlated with the number of functional traits in the mixtures. This finding further supports the diversity-productivity hypothesis of Tilman (2001) but at the intraspecific level (the higher the number of functional traits within lentil cultivar mixtures, the higher the biomass). Considering both, intraspecific and interspecific diversity, the highest total biomass in the system was recorded when both were maximised. Whether this result was driven by niche-complementarity effects or by the diversity of the microbiota, is still to be tested.

## Credit author statement

Elisa Lorenzetti: Conceptualization, Data curation, Formal analysis,

Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. Federico Leoni: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Paolo Bärberi: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision. Gilbert Koskey: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Funding

This work has received funding from “Legumes in biodiversity-based farming systems in Mediterranean basin” project (LEGU-MED) funded by PRIMA Foundation and from Scuola Superiore Sant’Anna, PhD Programme in Agrobiodiversity, who funded E.L.’s study grant.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

## Acknowledgements

The authors would like to thank Giacomo Nardi, Cristiano Tozzini, Fabio Taccini for their support in field trial management and sampling. We thank Rete Semi Rurali for the facilitation with many of the providers of the lentil genetic material, especially Bari CNR and ARSIAL. Our gratitude also goes to IPK Gatersleben, for University of Perugia, Catania University, Naples University (in the kind person of Professor Frusciantè) and the CNR Pontecagnano for the provision of genetic material. Finally, our appreciation also goes to Prof. Alessandra Turrini and Prof. Luciano Avio (Soil Microbiology Lab, University of Pisa) for their technical guidance in mycorrhiza assessment.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rhisph.2023.100824>.

## References

- Amossé, C., Jeuffroy, M.H., Celette, F., David, C., 2013. Relay-intercropped forage legumes help to control weeds in organic grain production. *Eur. J. Agron.* 49, 158–167. <https://doi.org/10.1016/j.eja.2013.04.002>.
- Bärberi, P., Moonen, A.C., 2020. Functional biodiversity for the provision of agroecosystem services. In: *Reconciling Agricultural Production with Biodiversity Conservation*. Burleigh Dodds Sci. Pub, pp. 101–146.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67/114810.18637/jss.v067.i01.
- Bedoussac, L., Journet, E.P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E.S., et al., 2015. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agron. Sustain. Dev.* 35, 911–935. <https://doi.org/10.1007/s13593-014-0277-7>.



- Bektas, H., Hohn, C.E., Waines, J.G., 2016. Root and shoot traits of bread wheat (*Triticum aestivum* L.) landraces and cultivars. *Euphytica* 212 (2), 297–311. <https://doi.org/10.1007/s10681-016-1770-7>.
- Bulgarelli, D., Garrido-Oter, R., Münch, P.C., Weiman, A., Dröge, J., Pan, Y., et al., 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17 (3), 392–403. <https://doi.org/10.1016/j.chom.2015.01.011>.
- Cardinale, M., Grube, M., Erlacher, A., Quehenberger, J., Berg, G., 2015. Bacterial networks and co-occurrence relationships in the lettuce root microbiota. *Environ. Microbiol.* 17 (1), 239–252. <https://doi.org/10.1111/1462-2920.12686>.
- Chamkhi, I., Cheto, S., Geistlinger, J., Zeroual, Y., Kouisni, L., Bargaz, A., Ghoulam, C., 2022. Legume-based intercropping systems promote beneficial rhizobacterial community and crop yield under stressing conditions. *Ind. Crops Prod.* 183, 114958 <https://doi.org/10.1016/j.indcrop.2022.114958>.
- Creissen, H.E., Jorgensen, T.H., Brown, J.K., 2016. Increased yield stability of field-grown winter barley (*Hordeum vulgare* L.) varietal mixtures through ecological processes. *Crop Protect.* 85, 1–8. <https://doi.org/10.1016/j.cropro.2016.03.001>.
- de Novais, C.B., Sbrana, C., da Conceição Jesus, E., Rouws, L.F.M., Giovannetti, M., Avio, L., et al., 2020. Mycorrhizal networks facilitate the colonization of legume roots by a symbiotic nitrogen-fixing bacterium. *Mycorrhiza* 30, 389–396. <https://doi.org/10.1007/s00572-020-00948-w>.
- Dobert, R.C., Blevins, D.G., 1993. Effect of seed size and plant growth on nodulation and nodule development in lima bean (*Phaseolus lunatus* L.). *Plant Soil* 148 (1), 11–19. <https://doi.org/10.1007/BF02185380>.
- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Scheu, S., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91 (2), 485–496. <https://doi.org/10.1890/08-2338.1>.
- Figueroa-Bustos, V., Palta, J.A., Chen, Y., Siddique, K.H., 2018. Characterization of root and shoot traits in wheat cultivars with putative differences in root system size. *Agronomy* 8 (7), 109. <https://doi.org/10.3390/agronomy8070109>.
- Ganugi, P., Masoni, A., Pietramellara, G., Benedettelli, S., 2019. A review of studies from the last twenty years on plant-arbuscular mycorrhizal fungi associations and their uses for wheat crops. *Agronomy* 9 (12), 840. <https://doi.org/10.3390/agronomy9120840>.
- Geren, H., Avcioglu, R., Soya, H., Kir, B., 2008. Intercropping of corn with cowpea and bean: biomass yield and silage quality. *Afr. J. Biotechnol.* 7 (22).
- Giovanetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>.
- Harinikumar, K.M., Bagyaraj, D.J., Mallesh, B.C., 1990. Effect of intercropping and organic soil amendments on native VA mycorrhizal fungi in an oasis. *Arid Land Res. Manag.* 4 (3), 193–197. <https://doi.org/10.1080/15324989009381248>.
- Hoeksema, J.D., Chaudhary, V.B., Gehring, C.A., Johnson, N.C., Karst, J., Koide, R.T., et al., 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol. Lett.* 13 (3), 394–407. <https://doi.org/10.1111/j.1461-0248.2009.01430.x>.
- Ingraffia, R., Amato, G., Frenda, A.S., Giambalvo, D., 2019. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N<sub>2</sub> fixation, N transfer, and growth in a wheat/faba bean intercropping system. *PLoS One* 14 (3), e0213672. <https://doi.org/10.1371/journal.pone.0213672>.
- Javaid, A., 2008. Allelopathy in mycorrhizal symbiosis in the Poaceae family. *Allelopathy J.* 21 (2), 207.
- Jensen, E.S., Carlsson, G., Hauggaard-Nielsen, H., 2020. Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: a global-scale analysis. *Agron. Sustain. Dev.* 40, 1–9. <https://doi.org/10.1007/s13593-020-0607-x>.
- Jie, W., Liu, X., Cai, B., 2013. Diversity of rhizosphere soil arbuscular mycorrhizal fungi in various soybean cultivars under different continuous cropping regimes. *PLoS One* 8 (8), e72898. <https://doi.org/10.1371/journal.pone.0072898>.
- Kabululu, M.S., Ojiewo, C., Oluoch, M., Maass, B.L., 2014. Cowpea intercrop mixtures for stable and optimal leaf and seed yields in a maize intercropping system. *Int. J. Veg. Sci.* 20 (3), 270–284. <https://doi.org/10.1080/19315260.2013.813889>.
- Koskey, G., Leoni, F., Carlesi, S., Avio, L., Barberi, P., 2022a. Exploiting plant functional diversity in durum wheat-lentil relay intercropping to stabilize crop yields under contrasting climatic conditions. *Agronomy* 12 (1), 210. <https://doi.org/10.3390/agronomy12010210>.
- Koskey, G., Avio, L., Turrini, A., Sbrana, C., Barberi, P., 2022b. Biostimulatory effect of vermicompost extract enhances soil mycorrhizal activity and selectively improves crop productivity. *Plant Soil* 1–17. <https://doi.org/10.1007/s11104-022-05783-w>.
- Koskey, G., Avio, L., Turrini, A., Sbrana, C., Barberi, P., 2023. Durum wheat-lentil relay intercropping enhances soil mycorrhizal activity but does not alter structure of arbuscular mycorrhizal fungal community within roots. *Agric. Ecosyst. Environ.* 357, 108696 <https://doi.org/10.1016/j.agee.2023.108696>.
- Layek, J., Das, A., Mitran, T., Nath, C., Meena, R.S., Yadav, G.S., et al., 2018. Cereal+ legume intercropping: an option for improving productivity and sustaining soil health. In: Meena, R., Das, A., Yadav, G., Lal, R. (Eds.), *Legumes for Soil Health and Sustainable Management*. Springer, Singapore. [https://doi.org/10.1007/978-981-13-0253-4\\_11](https://doi.org/10.1007/978-981-13-0253-4_11).
- Lazzaro, M., Costanzo, A., Barberi, P., 2018. Single vs multiple agroecosystem services provided by common wheat cultivar mixtures: weed suppression, grain yield and quality. *Field Crops Res.* 221, 277–297. <https://doi.org/10.1016/j.fcr.2017.10.006>.
- Lehmann, A., Barto, E.K., Powell, J.R., Rillig, M.C., 2012. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. *Plant Soil* 355, 231–250. <https://doi.org/10.1007/s11104-011-1095-1>.
- Leigh, J., Fitter, A.H., Hodge, A., 2011. Growth and symbiotic effectiveness of an arbuscular mycorrhizal fungus in organic matter in competition with soil bacteria. *FEMS Microbiol. Ecol.* 76 (3), 428–438. <https://doi.org/10.1111/j.1574-6941-2011.01066.x>.
- Lenth, Russell V., 2020. *emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.3.*
- Leoni, F., Hazrati, H., Fomsgaard, I.S., Moonen, A.C., Kudsk, P., 2021. Determination of the effect of co-cultivation on the production and root exudation of flavonoids in four legume species using LC-MS/MS analysis. *J. Agric. Food Chem.* 69 (32), 9208–9219. <https://doi.org/10.1021/acs.jafc.1c02821>.
- Leoni, F., Lazzaro, M., Ruggeri, M., et al., 2022. Relay intercropping can efficiently support weed management in cereal-based cropping systems when appropriate legume species are chosen. *Agron. Sustain. Dev.* 42, 75. <https://doi.org/10.1007/s13593-022-00787-3>.
- Li, B., Li, Y.Y., Wu, H.M., Zhang, F.F., Li, C.J., Li, X.X., et al., 2016. Root exudates drive inter-specific facilitation by enhancing nodulation and N<sub>2</sub> fixation. *Proc. Natl. Acad. Sci. USA* 113 (23), 6496–6501. <https://doi.org/10.1073/pnas.1523580113>.
- Liu, Y.C., Qin, X.M., Xiao, J.X., Tang, L., Wei, C.Z., Wei, J.J., Zheng, Y., 2017. Intercropping influences component and content change of flavonoids in root exudates and nodulation of Faba bean. *J. Plant Interact.* 12 (1), 187–192. <https://doi.org/10.1080/17429145.2017.1308569>.
- Lopes, T., Hatt, S., Xu, Q., Chen, J., Liu, Y., Francis, F., 2016. Wheat (*Triticum aestivum* L.)-based intercropping systems for biological pest control. *Pest Manag. Sci.* 72 (12), 2193–2202. <https://doi.org/10.1002/ps.4332>.
- Lorenzetti, E., Carlesi, S., Barberi, P., 2022. Mixtures of commercial lentil cultivars show inconsistent results on agronomic parameters but positive effects on yield stability. *Agronomy* 12 (9), 2107. <https://doi.org/10.3390/agronomy12092107>.
- Lorenzetti, E., Dell'Acqua, M., Carlesi, S., Barberi, P., 2023. 'Unlocking Genetic Diversity for Low Input Systems in a Changing Climate: Participatory Characterization and Genome-wide Association Study of Lentil Landraces'. *agRxiv*. CABI. <https://doi.org/10.31220/agriRxiv.2023.00198>. PREPRINT.
- Mauger, S., Ricono, C., Mony, C., Chable, V., Serpouly, E., Biget, M., et al., 2021. Differentiation of endospheric microbiota in ancient and modern wheat cultivar roots. *Plant-Environment Interactions* 2 (5), 235–248. <https://doi.org/10.1002/pe13.10062>.
- Mazeroll, M.J., 2020. AICcmoavg: Model Selection and Multimodel Inference Based on (Q)AIC(c). R Package Version 2.3-1. <https://cran.r-project.org/package=AICcmoavg>.
- Moonen, A.C., Barberi, P., 2008. Functional biodiversity: an agroecosystem approach. *Agric. Ecosyst. Environ.* 127 (1–2), 7–21. <https://doi.org/10.1016/j.agee.2008.02.013>.
- Newton, A.C., 2016. Exploitation of diversity within crops—the key to disease tolerance? *Front. Plant Sci.* 7, 665. <https://doi.org/10.3389/fpls.2016.00665>.
- Njeru, E.M., Avio, L., Sbrana, C., Turrini, A., Bocci, G., Barberi, P., Giovannetti, M., 2014. First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. *Agron. Sustain. Dev.* 34, 841–848. <https://doi.org/10.1007/s13593-013-0197-y>.
- Njeru, E.M., Bocci, G., Avio, L., Sbrana, C., Turrini, A., Giovannetti, M., Barberi, P., 2017. Functional identity has a stronger effect than diversity on mycorrhizal symbiosis and productivity of field grown organic tomato. *Eur. J. Agron.* 86, 1–11. <https://doi.org/10.1016/j.eja.2017.02.007>.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 158. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3). IN18.
- R Core Team, 2021. *R: A Language and Environment for Statistical Computing*. R Found. Stat. Comput. <https://www.r-project.org/>.
- Rinaldo, M., Carlesi, S., Costanzo, A., Barberi, P., 2020. Functional diversity of cover crop mixtures enhances biomass yield and weed suppression in a Mediterranean agroecosystem. *Weed Res.* 60 (1), 96–108. <https://doi.org/10.1111/wre.12388>.
- Renard, D., Tilman, D., 2021. Cultivate biodiversity to harvest food security and sustainability. *Curr. Biol.* 31 (19), R1154–R1158.
- Roy, A., Ghosh, S., Kundagrami, S., 2019. Nodulation pattern and its association with seed yield in chickpea (*Cicer arietinum* L.) germplasm. *Indian J. Agric. Res.* 53 (2), 172–177.
- Schnitzer, S.A., Klironomos, J.N., HilleRisLambers, J., Kinkel, L.L., Reich, P.B., Xiao, K., et al., 2011. Soil microbes drive the classic plant diversity–productivity pattern. *Ecol.* 92 (2), 296–303. <https://doi.org/10.1890/10-0773.1>.
- Sessitsch, A., Brader, G., Pfaffenbichler, N., Gusenbauer, D., Mitter, B., 2018. The contribution of plant microbiota to economy growth. *Microb. Biotechnol.* 11 (5), 801–805. <https://doi.org/10.1111/1751-7915.13290>.
- Talukdar, N.C., Germida, J.J., 1994. Growth and yield of lentil and wheat inoculated with three *Glomus* isolates from Saskatchewan soils. *Mycorrhiza* 5, 145–152. <https://doi.org/10.1007/BF00202347>.
- Tilman, D., 2001. Functional diversity. *Encycl. Biodivers.* 3 (1), 109–120.
- Turrini, A., Bedini, A., Loor, M.B., Santini, G., Sbrana, C., Giovannetti, M., Avio, L., 2018. Local diversity of native arbuscular mycorrhizal symbionts differentially affects growth and nutrition of three crop plant species. *Biol. Fertil. Soils.* 54, 203–217. <https://doi.org/10.1007/s00374-017-1254-5>.
- Valente, J., Gerin, F., Le Gouis, J., Moëne-Loccoz, Y., Prigent-Combaret, C., 2019. Ancient wheat varieties have a higher ability to interact with plant growth-promoting rhizobacteria. *Plant Cell Environ.* 43, 246–260. <https://doi.org/10.1111/pce.13652>.
- Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11 (3), 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>.



- Vandenberg, A., Kiehn, F.A., Vera, C., Gaudiel, R., Buchwaldt, L., Dueck, S., E Slinkard, A., 2002. CDC Robin lentil. *Can. J. Plant Sci.* 82 (1), 111–112. <https://doi.org/10.4141/P01-003>.
- Vandermeer, J.H., 1992. *The Ecology of Intercropping*. Cambridge university press.
- Wuest, S.E., Peter, R., Niklaus, P.A., 2021. Ecological and evolutionary approaches to improving crop variety mixtures. *Nat. Ecol. Evol.* 5 (8), 1068–1077. <https://doi.org/10.1038/s41559-021-01497-x>.
- Xiao, X., Han, L., Chen, H., Wang, J., Zhang, Y., Hu, A., 2023. Intercropping enhances microbial community diversity and ecosystem functioning in maize fields. *Front. Microbiol.* 13, 1084452 <https://doi.org/10.3389/fmicb.2022.1084452>.
- Yang, L.N., Pan, Z.C., Zhu, W., Wu, E.J., He, D.C., Yuan, X., et al., 2019. Enhanced agricultural sustainability through within-species diversification. *Nat. Sustain.* 2 (1), 46–52. <https://doi.org/10.1038/s41893-018-0201-2>.
- Zuur, Alain F., 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY.