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REGULAR ARTICLE

Species identity, rather than species mixtures, drives cover crop effects on nutrient partitioning in unfertilized agricultural soil

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Abstract

Aims Previous cover crop studies mainly focused on the links between plant uptake and soil fertility, and there is a clear knowledge gap regarding the role of microbes in these processes. Our aim was then to better understand the effects of plant mixtures (versus monoculture) and the specific effects of each plant species on nitrogen (N) and phosphorus (P) partitioning between plant, soil, and more particularly microbial pools.

Methods Monocultures and mixtures composed of black oat, field pea and Indian mustard were grown during two months in a greenhouse. The concentrations of carbon (C), N and P were measured in both plant and microbial biomass at final harvest, together with soil available N and P.

Results Overall, our findings highlight stronger selection effect (i.e., presence of key species) rather than complementarity effects (i.e., species mixture) to affect the measured parameters. The presence of pea increased the biomass production of oat and mustard, as well as the nutrient concentration of oat, whereas pea P concentration decreased in presence of oat and mustard N and P concentrations were negatively impacted respectively by the presence of oat and pea. We also observed a strong competition between plants and microbes for both soil N and P.

Conclusions The oat-pea and the oat-pea-mustard mixtures represented the best compromise between biomass production, nutrient storage and biomass C:N ratio, thus insuring a good organic matter decomposition and nutrient provision for the following main crop.

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Introduction

Conventional field cropping systems often require high fertilizer inputs to meet yield and economic goals. However, since mineral fertilizer stocks tend to get depleted worldwide (Liu et al. 2008; Cordell and Neset 2014; Chowdhury et al. 2017) and their production is high-energy demanding (Pimentel et al. 1973; Galloway 1998; Hamza and Anderson 2005; Erisman et al. 2008), conventional cropping systems might become less and less sustainable. Furthermore, the lack of soil cover between two crops promotes erosion, aggregate breakdown and compaction, since soil is no longer protected from weathering (Bradford and Huang 1994; Nearing et al. 2005; Chen et al. 2014). Environmental damages were also reported in conventional cropping systems, including impoverishment of soil quality (Fliessbach et al. 2007), diminution of soil carbon sequestration (Paustian et al. 1997), and reduced resistance and resilience to drought (Kremen and Miles 2012).

By contrast to the open nutrient cycle (i.e., massive inputs and outputs) of croplands, natural ecosystems generally have a better nutrient-use efficiency, with nutrients taken up by plants and returned to the soil in mineral forms after organic matter decomposition (Mariotte et al. 2018). In agro-ecosystems, one way to improve nutrient cycling, sustainably maintain soil fertility and protect soil structure is to grow cover crops in rotation with the main crops. Cover crops consist in a monoculture or mixture of particular species that are not harvested and can maintain soil cover, reduce nutrient losses and improve nitrogen (N) nutrition when including N-fixing legume species (Mariotte et al. 2018; Wittwer et al. 2017). Species used in cover crop mixtures predominantly belong to the three following plant families: Fabaceae, Poaceae and Brassicaceae. As mentioned above, legumes (Fabaceae) are particularly useful because they are efficient N fixers and may in some cases improve N uptake for neighboring plants (Dubach and Russelle 1994; Temperton et al. 2007). Additionally, they are able to increase phosphorus (P) availability for other plants, due to the release of organic anions or acids in the rhizosphere (Nuruzzaman et al. 2005; Hinsinger et al. 2011). Thin, dense and fibrous root systems of grasses (Poaceae) improve the soil structure at the surface layer (Gyssels et al. 2005). Furthermore, grasses contribute efficiently to the increase of soil organic matter content (Okubo et al. 2016) and to the provision of habitats for soil organisms (Magdoff and Weil 2004; Brady and Weil 2016). Brassicaceae are used to cover the soil and prevent weeds' germination through competition, due to their fast growth, but also because of their allelopathic effects (Gfeller et al. 2018; Norsworthy 2003; Al-Sherif et al. 2013). They can also improve deep soil structure through the action of their pivoting roots (Hamza and Anderson 2005) and catalyze the release of organic P, which become available for both microbial and plant communities (Jones et al. 2009; Hunter et al. 2014).

Cover crop mixtures are generally more productive (Wendling et al. 2017) and more resistant to environmental stresses (Kremen and Miles 2012; Lin 2011; Tengö and Belfrage 2004) than monocultures. This is due to two mechanisms, the "selection effect" of one species or functional group with particular functional traits and associated functions, and/or the "niche complementarity effect" with a greater range of functional traits providing opportunities for a more efficient use of resources (Díaz and Cabido 2001). As previously shown, improving biomass production (Díaz and Cabido 2001), N and P uptake (Kahmen et al. 2006; Oelmann et al. 2011), soil cover (Spehn et al. 2005), microbial biomass (Zak et al. 2003) or organic matter decomposition (Spehn et al. 2005) can not only be achieved through increased species diversity but also through the presence of key plant functional groups within multi-species mixtures. In cover crop mixtures, the challenge is then to find the best combination of selection and resource-use complementarity effects depending on the targeted needs, whether it is to increase biomass production, nutrient uptake or nutrient sequestration in soil and microbes.

While previous studies already investigated the role of cover crop species in improving soil quality and fertility (Doran and Smith 1991; Dabney et al. 2001; Delgado et al. 2007), much less effort has been dedicated in evaluating the role of plant-microbe interaction. Soil microbes increase organic matter decomposition rate and nutrient release that can be used by plants (Bardgett et al. 2005). They play a major role in several biogeochemical cycles and it was estimated that mycorrhizal fungi and nitrogen-fixing bacteria are responsible for 5 to 80% of N, and up to 75% of P acquired by plants

annually (van der Heijden et al. 2008). For example, N enters ecosystems via atmospheric N_2 fixation by nitrogen-fixing bacteria and thus improves N availability (Moore 1974; Zahran 1999). Apart from nutrient recycling, microbes are also efficient in sequestrating both N and P. For example, they are able to accumulate excess of P in favorable conditions, thus providing a temporary storage of nutrients (Tang et al. 2014). Furthermore, microbes usually have better nutrient acquisition capabilities than plants (Hodge et al. 2000; Richardson and Simpson 2011).

In this pot experiment, we aimed to compare the effects of three functionally distinct cover crop species (black oat (*Avena strigosa* Schreb.), field pea (*Pisum sativum* L.) and Indian mustard (*Brassica juncea* (L.) Czern.)) on N and P distribution within the plant-soil-microbe system when grown in mixture versus monoculture. First, we hypothesized that functional diversity in cover crops promotes resource-use complementarity and facilitation. Thus, higher biomass in the pot, together with higher nutrient concentrations in both plants and microbes are expected in cover crops mixtures compared to respective monocultures. Second, we expected species-specific effects on biomass production and nutrient partitioning within the plant-soil-microbe system. More particularly, we predicted that the presence of oat will increase microbial biomass, the presence of pea will be beneficial for plant biomass production and nutrient concentration through atmospheric N fixing, and the presence of mustard will reduce cover crop productivity (due to allelopathy effects) but increase soil P availability.

Material and methods

Greenhouse experiment

The experiment was carried out in a greenhouse at the research station Agroscope (Changins site) in Nyon, Switzerland (46°23′58.3”N, 6°14′9.0″E, 426 m a.s.l.), starting from the 12th of March until the 12th of May 2018. Temperature was controlled to ensure optimal growing conditions, 20 °C during the day (from 6 am to 9 pm) and 15 °C during the night (from 11 pm to 4 am) with two hours of transition. The light was also controlled by using 14 lamps of 400 W m^{-2}, which were switched on between 7 am and 7 pm when natural light intensity dropped below 250 W m^{-2}.

Soil preparation and potting protocol

The soil used for the pot experiment was sampled in a loamy clay field in Changins (46°23′57.8”N, 6°14′22.6″E), a soil commonly found within the Lake Geneva area and classified as cambisol (IUSS Working Group WRB 2006). The collected soil was sieved (1 cm mesh) to remove stones, gravels and coarse organic debris from previous crops, and homogenized. This soil was characterized by 29.4% clay, 28.5% fine silt, 14.8% coarse silt, 16.6% fine sand, 10.7% coarse sand, a pH of 7.9 and 6% organic matter. At the beginning of the experiment, the soil contained on average 11.73 mg kg^{-1} nitrate, 0.84 mg kg^{-1} ammonium and 20 mg kg^{-1} available phosphorus (Olsen method). No fertilizer was applied for this experiment and the soil was considered as nutrient-limited due to the low soil N and P availability.

The experiment was carried out in pots of 12 L (0.25 m diameter × 0.25 m height). To guarantee identical initial conditions for plant growth, each pot was filled up to the top with 12 kg of soil. Then, the top-layer of approximately 1 cm was removed, the soil surface was slowly watered with 200 mL of tap water to prepare the seedling bed, then the seeds were uniformly distributed on the wet surface and covered by the previously removed top-layer. All pots were watered to 70% of water holding capacity (WHC, calculated following (Feodoroff and Betremieux 1964), which corresponded to 167 mL water kg dry $soil^{-1}$. During the experiment, one pot of each cover crop treatment was weighed twice a week to determine the required amount of water to maintain 70% WHC. Pot weight was corrected to consider the additional weight of the fresh biomass over the 2-month experiment, estimated according to the results of Wendling et al. (2016). Stakes were installed around the pots to allow plants to stay upright, as in field conditions.

Cover crop treatments

The experiment was carried out with three species commonly used as cover crops in Switzerland: field pea (Fabaceae; hereafter called ‘pea’), black oat (Poaceae; hereafter called ‘oat’) and Indian mustard (Brassicaceae; hereafter called ‘mustard’). Each plant species was grown in monoculture (oat, pea, mustard), in two-species mixture (oat-pea, oat-mustard, mustard-pea) and in three-species mixture (oat-pea-mustard). Each of the seven treatments was replicated 6 times, for a total of 42 pots. The 42 pots were distributed on 6 tables

in the greenhouse, the location and orientation of blocks within the greenhouse were randomly moved every two weeks to homogenize growing conditions, forming 6 statistical blocks of 7 treatments each.

The standard sowing densities (SSD) for monoculture were 400 seeds m^{-2} (12 g m^{-2}) for oat, 150 seeds m^{-2} (22.5 g m^{-2}) for pea and 500 seeds m^{-2} (3.5 g m^{-2}) for mustard, which correspond to the optimal densities used for short-term cover crops (Wendling et al. 2016, 2017). For the two- and three-species mixtures, the proportions of the standard densities and ratios of each species were chosen to obtain the highest biomass production based on i) the mixture yields and the competitiveness results obtained by Wendling et al. (2016, 2017), mustard being the most competitive and pea the least competitive species, and ii) the advice received by two advisory companies (Arvalis, Paris, France; Proconseil, Lausanne, Switzerland) with practical expertise in cover crop cultivation. Thinning occurred after germination of the seeds in order to obtain the desired number of seedlings per species in each pot. The number of individuals per species and pot in each cover crop treatment and the corresponding SSD proportion are given in Table 1.

Plant biomass harvest and chemical analysis

After 2 months, plants reached the peak of biomass production and the shoot biomass was harvested by cutting plants 2 cm above soil level. The lower part of the stem (2 cm) of each plant species was previously marked with a different colored duct tape. The block of soil was then extracted from each pot and cut vertically into two equal parts. One half remained intact and was used for soil and microbial nutrient analysis (see below). The second half was destructed to determine root biomass; it was immersed in a bucket of water during about 30 min, then washed with a shower head to remove the soil particles from the roots. The root system of each individual plant was kept separated and the colored duct tape allowed to sort the individuals into species. Nevertheless, a low amount of root material was lost when disentangling the individuals of different species. Finally, the remaining 2 cm of stems were cut and added to the shoot biomass of each species. The fresh shoot and root biomass of each species was kept separated, dried at 65 °C during 4 days and weighed. Shoot and root biomasses (g) per pot (i.e., soil surface area of 0.05 m^2) were then converted in [t/ha].

Table 1 Number of individuals per species in each pot and proportion (%, in parentheses) of the standard sowing densities (SSD: oat = 400 seeds m^{-2}, pea = 150 seeds m^{-2}, mustard = 500 seeds m^{-2}) for the different cover crop treatments

Cover crop treatments	Oat	Pea	Mustard	Total
Monocultures				
Oat	20 (100)			20 (100)
Pea		7 (100)		7 (100)
Mustard			25 (100)	25 (100)
Two-species mixtures				
Oat-Pea	8 (40)	5 (71)		13 (111)
Oat-Mustard	13 (65)		8 (32)	21 (97)
Mustard-Pea		6 (86)	6 (24)	12 (110)
Three-species mixture				
Oat-Pea-Mustard	6 (30)	4 (57)	5 (20)	15 (107)

The dried root and shoot samples collected for each species in each pot (i.e., pool of the individuals of the same species per plot) were ground using a ball mixer mill MM200 (Retsch, Haan, Germany) to a fine powder prior to chemical analyses. Plant carbon (hereafter called 'shoot C', 'root C', and 'plant C' for the sum of shoot and root C) and nitrogen (hereafter called 'shoot N', 'root N' and 'plant N' for the sum of shoot and root N) concentrations were determined by thermal combustion using an Elemental analyser EA 1110 (EA Consumables Inc., Pennsauken, USA). Plant phosphorus (hereafter called 'shoot P', 'root P', and 'plant P' for the sum of shoot and root N) was extracted from 0.5 g of ground powder (separately for shoot and root biomass) that was ashed (4 h at 450 °C) and dissolved in 20 mL of 0.5 M H_2SO_4 for 18 h. The P concentration in filtered extracts was then determined colorimetrically using the malachite green phosphate assay kit (MAK307, Sigma-Aldrich, Saint-Louis, MO, USA). Plant nutrient concentrations were expressed in mg g^{-1} dry weight.

Soil respiration

Immediately after the shoot biomass harvest, soil respiration was measured using a LI-8100A automated soil CO_2 flux system equipped with a LI-8100-102 chamber (LI-COR Biosciences,

Lincoln, NE, USA) to obtain instantaneous soil CO_2 efflux measurements (Liang et al. 2004). An insulated funnel was tightly fixed on the pot surface and connected to the 10-cm survey chamber. Each measurement

lasted 3 min, with a dead band of 30 s. The increase of CO_2 concentration inside the known volume of the chamber and the funnel was used to calculate the CO_2 flux, using an exponential regression and correcting for atmospheric pressure, temperature and soil surface.

Soil and microbial biomass chemical properties

The second half of each soil block was used for chemical analysis. Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined after extraction of 5 g of fresh soil with 30 mL 1 M KCl, using an automated analyzer (AA3 HR Autoanalyser, Seal Analytical, UK).

For the determination of microbial biomass C and N (hereafter called 'microbial C' and 'microbial N', respectively), pairs of 5 g of fresh soil samples were weighed and one sample was immediately extracted with 25 mL 0.5 M K_2SO_4, whereas the other sample was first fumigated with chloroform during 1 day to kill microbes and then extracted with the same solution as for the unfumigated sample (Jenkinson 1988). Total C and N concentrations in unfumigated and fumigated samples were analysed by a TOC/TN analyser (Total organic carbon analyser TOC-V, Shimadzu, Japan). To determine the soil available P and the microbial biomass P (hereafter called 'soil P' and 'microbial P'), pairs of 3 g of fresh soil (fumigated and unfumigated) were extracted with 40 ml of 0.5 M $NaHCO_3$. Soil P was analyzed colorimetrically (spectrophotometer at 890 nm) using the ammonium molybdate reagent (Olsen et al. 1954). Microbial C, microbial N and microbial P were estimated as the differences between the concentrations of C, N and P in fumigated and unfumigated samples and were corrected by dividing values by the extractability factors of 0.45 for C (Vance et al. 1987), 0.54 for N (Brookes et al. 1985) and 0.4 for P (Brookes et al. 1982). Soil and microbial nutrient contents were expressed as mg kg^{-1} dry soil (oven-dried at 105 °C for 24 h).

Statistical analysis

All analyses were carried out with R version 3.4.2 (R Development Core Team 2017). Data were log or square root transformed when necessary to meet the assumptions of normality and homoscedasticity.

First, we tested the cover crop treatments effects on the measured variables using a one-way ANOVAs with the 7 cover crop treatments as explanatory variables, followed by post hoc Tukey tests, specifying 'block' as random factor. Second, we tested the presence versus absence effects of a particular species on the measured variables at the community level by using one-way ANOVAs specifying 'block' as random factor. Third, we tested the effect of the different neighboring species on the plant biomass, C, N and P at the crop species level (oat, pea, mustard) by using one-way ANOVAs specifying 'block' as random factor.

Finally, a principal component analysis (PCA) using vegan package. The analysis was carried out on the full matrix of data (including total plant biomass production, plant C, plant N, plant P, plant C:N ratio, soil respiration, soil NH_4^+, soil NO_3^-, soil P, microbial C, microbial N and microbial P) in order to determine how the different cover crop treatments drive nutrient dynamic in the different pools (plant, soil and microbes).

Results

All the measured variables were significantly affected by the cover crop treatment (Table 2). Root and shoot biomass were the highest in cover crop treatments that contained pea (pea, oat-pea, mustard-pea, oat-mustard-pea). Indeed, the plant biomass was on average 72% higher in the cover crop treatments with pea compared to those without pea (Table 3). By contrast, the monoculture of mustard was the least productive, and overall, the plant biomass decreased by 22% in the presence of mustard in the cover crop (Table 3).

The shoot and root N concentrations of the cover crop were also highest in presence of pea, especially in monoculture and in the mixture with mustard, while they were relatively low in the treatments without pea (Table 2). Overall, the plant N concentration was 244% higher in presence of pea (Table 3). When considering the total amount of N stored in plants, the monoculture of pea stored the highest amount, corresponding on average to 380 kg N ha^{-1} (shoot and root, Table 2). The shoot P concentration was the highest for pea monoculture and the lowest for oat monoculture. For the root P concentration, the mustard monoculture had the highest value and the oat monoculture the lowest (Table 2). When considering the total amount of P stored in plants, the monoculture of pea stored the highest amount, corresponding on average to 22 kg P

Table 2 Effects of the seven cover crop treatments on all measured variables. Data are mean values ± SE; $n = 6$. Significant effects of the cover crop treatment are indicated with * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$ (one-way ANOVAs). Different letters indicate significant differences between treatments with a > b > c > d > e. C = carbon, N = nitrogen, P = phosphorus

	Units	Oat	Pea	Mustard	Oat-Pea	Oat-Mustard	Mustard-Pea	Oat-Pea-Mustard	Anova test
Shoot Biomass	t DM ha^{-1}	5.68 ± 0.15 c	8.67 ± 0.16 a	3.46 ± 0.26 e	8.27 ± 0.10 a	4.74 ± 0.15 d	8.26 ± 0.10 a	7.41 ± 0.18 b	***
Shoot C	mg $(g\ DM)^{-1}$	406 ± 1 bc	423 ± 2 a	373 ± 3 d	416 ± 1 abc	400 ± 2 c	429 ± 3 a	422 ± 8 a	*
Shoot N	mg $(g\ DM)^{-1}$	7 ± 0.1 d	33 ± 1.5 a	8 ± 0.1 c	23 ± 0.8 b	8 ± 0.2 cd	30 ± 0.8 a	23 ± 0.4 b	***
Shoot P	mg $(g\ DM)^{-1}$	1.0 ± $6e^{-2}$ c	1.8 ± 0.6 a	1.3 ± 0.2 bc	1.3 ± 0.1 abc	1.2 ± 0.2 bc	1.4 ± $7e^{-2}$ ab	1.2 ± 0.1 bc	***
Shoot C:N		57.85 ± 0.99 a	12.72 ± 0.63 d	44.89 ± 0.90 b	18.37 ± 0.65 c	52.89 ± 1.93 a	14.09 ± 0.31 d	18.30 ± 0.40 c	**
Total Shoot N	kg $N.ha^{-1}$	39 ± 1 d	291 ± 11 a	29 ± 1 e	188 ± 6 c	36 ± 1 d	252 ± 7 b	171 ± 4 c	***
Total Shoot P	kg $P.ha^{-1}$	5.8 ± 0.3 c	15.2 ± 2.0 a	4.3 ± 0.2 c	10.4 ± 0.4 b	5.8 ± 0.4 c	11.7 ± 0.3 ab	8.8 ± 0.4 b	***
Root Biomass	t DM ha^{-1}	1.85 ± 0.10 bc	2.52 ± 0.15 a	0.90 ± 0.08 d	2.28 ± 0.07 ab	1.64 ± 0.15 c	2.29 ± 0.18 ab	2.17 ± 0.13 ab	***
Root C	mg $(g\ DM)^{-1}$	391 ± 6 bc	379 ± 11 c	402 ± 15 abc	414 ± 2 ab	411 ± 6 ab	415 ± 1 ab	415 ± 3 a	***
Root N	mg $(g\ DM)^{-1}$	7 ± 0.3 d	36 ± 1.4 a	12 ± 0.8 c	26 ± 0.4 b	9 ± 0.4 d	35 ± 0.8 a	25 ± 0.3 b	***
Root P	mg $(g\ DM)^{-1}$	0.5 ± 0.1 e	2.7 ± 0.2 b	3.4 ± 0.3 a	1.7 ± 0.2 cd	1.6 ± 0.1 d	2.9 ± 0.1 ab	2.3 ± 0.1 bc	***
Root C:N		57.78 ± 1.92 a	10.55 ± 0.16 e	32.57 ± 1.67 c	16.12 ± 0.29 d	47.28 ± 2.50 b	11.72 ± 0.27 e	16.37 ± 0.21 d	***
Total Root N	kg $N.ha^{-1}$	13 ± 1 cd	91 ± 7 a	11 ± 1 d	59 ± 2 b	14 ± 1 c	81 ± 4 a	55 ± 4 b	***
Total Root P	kg $P.ha^{-1}$	0.9 ± 0.2 d	6.8 ± 0.5 a	3.0 ± 0.3 c	3.9 ± 0.3 bc	2.6 ± 0.3 c	6.5 ± 0.1 a	4.9 ± 0.4 bc	***
Soil NH_4^+	mg kg^{-1}	1.21 ± 0.12 a	0.76 ± 0.06 b	0.65 ± 0.02 b	0.92 ± 0.10 ab	0.90 ± 0.08 ab	0.92 ± 0.09 ab	0.91 ± 0.07 ab	**
Soil NO_3^-	mg kg^{-1}	5.92 ± 0.91 b	14.41 ± 4.81 ab	16.39 ± 1.32 ab	21.05 ± 1.73 a	5.39 ± 0.71 b	15.48 ± 5.13 ab	14.55 ± 3.99 ab	*
Soil P	mg kg^{-1}	12.98 ± 0.15 a	10.26 ± 0.22 b	12.36 ± 0.51 a	11.62 ± 0.19 ab	12.70 ± 0.95 a	11.58 ± 0.10 ab	12.17 ± 0.13 ab	**
Soil respiration	µmol $CO_2.s^{-1}$ kg^{-1}	16 ± 3 d	49 ± 7 a	24 ± 2 cd	39 ± 3 abc	26 ± 4 bcd	49 ± 2 a	41 ± 6 ab	***
Microbial C	mg kg^{-1}	364 ± 71 a	299 ± 13 ab	219 ± 34 ab	297 ± 24 ab	357 ± 43 a	376 ± 19 a	183 ± 62 b	***
Microbial N	mg kg^{-1}	48 ± 3 ab	35 ± 3 bc	50 ± 3 a	32 ± 1 c	61 ± 4 a	46 ± 3 abc	51 ± 4 a	*
Microbial P	mg kg^{-1}	16 ± 1 ab	13 ± 1 ab	18 ± 2 a	11 ± 1 ab	16 ± 3 ab	10 ± 1 b	11 ± 1 b	***

Table 3 Significant results of the presence/absence effect of each cover crop species on the measured variables (one-way ANOVAs). *F*-values and associated *P*-values (with the respective symbols * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$) are indicated. The species effect (%), calculated as [(value in presence – value in absence) / value in absence × 100], corresponds to the relative increase or decrease of a measured variable due to the presence of a target species in cover crop treatments. C = carbon, N = nitrogen, P = phosphorus

	Oat			Pea			Mustard		
	F	*P*	Effect	*F*	*P*	Effect	*F*	*P*	Effect
Plant biomass				276.8	***	71.9	23.3	***	−22.1
Plant C				5.7	*	3.3			
Plant N	91.1	***	−37.4	379.7	***	243.5			
Plant P	11.8	**	−18.1	8.7	**	21.9			
Plant C:N	196.1	***	58.4	1228.7	***	−69.3	7.8	**	8.7
Soil NH_4^+	9.0	**	28.2						
Soil NO_3^-				6.7	*	77.7			
Soil P	8.1	**	8.6	11.2	**	−10.1			
Soil respiration	10.4	**	−24.3	50.8	***	104			
Microbial C									
Microbial N				33.2	***	−23.7	34	***	36
Microbial P				22.2	***	−32.3			

ha^{-1} (shoot and root, Table 2). When considering the species effect, plant P increased by 22% in the presence of pea, while it decreased by 18% in the presence of oat (Table 3). With respect to the shoot and root C:N ratios, values were below 19 in the presence of pea, and above 32 in the absence of pea (Table 2). Overall, plant C:N ratio decreased in the presence of pea (−69%), while it increased in the presence of oat (+58%) and mustard (+9%) (Table 3).

Soil ammonium (NH_4^+ and nitrate NO_3^-) were significantly affected by the cover crop treatment (Table 2), with a positive effect of oat presence on NH_4^+ (+28%) and a positive effect of pea presence on NO_3^- (+78%) (Table 3). Soil P was slightly affected by the cover crop treatment, with an increase in the presence of oat (+9%) and a decrease in the presence of pea (−10%) (Table 3).

Soil respiration was highest in all the cover crops that contained pea (Table 2) and indeed, in the presence of pea soil respiration increased by 104%, while it decreased by 24% in the presence of oat (Table 3). Albeit microbial C was significantly influenced by the cover crops treatment with the lowest value observed in the three-species mixture (Table 2), it was not specifically influenced by the presence of one of the three crop species (Table 3). In contrast, microbial N and P significantly decreased in the presence of pea (−24% and − 32%, respectively), whereas in the presence of mustard microbial N increased (+36%) (Table 3) and microbial P tended to be higher (Table 2, Fig. 1).

The scatter plot of the principal component analysis (Fig. 1) showed that the first two axes represented 55.3% of the variance. Along axis 1, there was a clear opposition between cover crops treatments containing pea (on the right side) and those without pea (on the left side), while the three-species mixture Oat-Mustard-Pea had a more central position. The presence of pea was associated with high values of plant biomass, plant N, plant P and soil respiration. Plant C and soil NO_3^- were also correlated with the presence of pea, but to a lesser extent. On the opposite side, cover crop treatments without pea were associated with high values of plant C:N ratio, microbial N and soil P. These cover crops without pea spread also along axis 2, those with monoculture of mustard being associated with higher microbial P and those with monoculture of oat being associated with higher soil NH_4^+.

Species-specific effects were found for the different variables measured in the cover crop mixtures (Table 4). Regarding oat, lowest values were recorded in monoculture for all variables, whereas the combination with

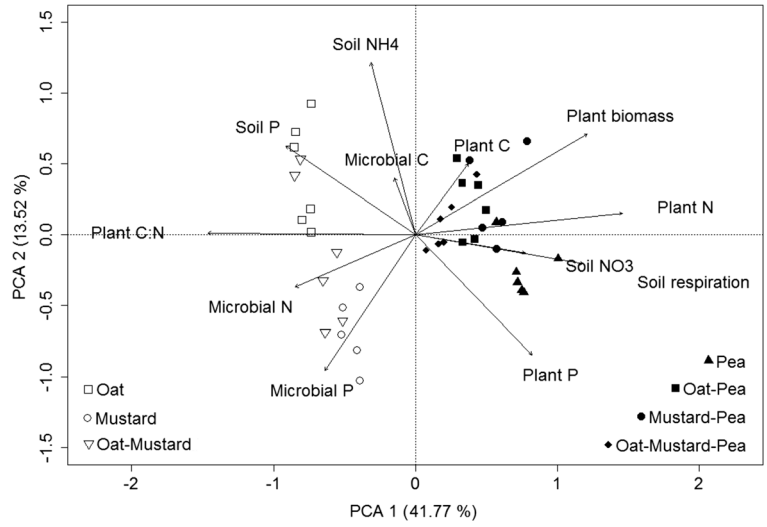

Fig. 1 Principal component analysis (PCA) including plant biomass (shoot + root), plant nutrient content (Plant N, Plant P and Plant C:N), microbial nutrient content (Microbial C, Microbial N and Microbial P), soil respiration and soil available nutrient content (Soil NH_4^+, Soil NO_3^- and Soil P) for the 7 cover crop treatments. Variance explained by each principal component is shown in parentheses. Black filled symbols highlight cover crop treatments with pea by comparison to open symbols which refers to treatments without pea

mustard induced the highest biomass production per individual and the three-species mixture yielded higher plant C, N and P concentrations. Pea produced more biomass per individual in monoculture than in mixture, but its C content was higher when growing with other species, with highest values recorded in three-species mixture. Furthermore, higher N and P contents for this species were observed in monoculture and when growing in mixture with mustard. Higher biomass production of mustard was recorded in three-species mixture and in

Table 4 Plant biomass per individual, carbon (C), nitrogen (N) and phosphorus (P) concentrations in plant tissues for the three cover crop species in monoculture and in mixtures with other species. Data are mean values ± SE; n = 6. Significant effects of the cover crop associated with oat, pea and mustard on biomass and nutrient contents are indicated with * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$ (one-way ANOVAs). Different letters indicate differences between combinations with a > b > c

	Plant biomass	Plant C	Plant N	Plant P
	g DM per individual	mg g DM^{-1}	mg g DM^{-1}	mg g DM^{-1}
Oat				
Monoculture	1.82 ± 0.06 c	402.1 ± 2.0 b	6.06 ± 0.09 c	0.90 ± 0.02 c
Mixture with Pea	2.54 ± 0.12 ab	407.4 ± 0.7 b	12.29 ± 0.40 b	1.80 ± 0.16 b
Mixture with Mustard	2.74 ± 0.09 a	408.0 ± 2.0 b	8.04 ± 0.24 c	1.20 ± 0.06 c
Three-species mixture	2.25 ± 0.13 b	421.9 ± 1.0 a	15.16 ± 0.48 a	2.53 ± 0.19 a
Anova test	***	***	***	***
Pea				
Monoculture	7.83 ± 0.09 a	413.3 ± 1.1 c	34.09 ± 1.23 ab	1.96 ± 0.20 a
Mixture with Oat	5.85 ± 0.13 c	421.1 ± 1.6 b	31.80 ± 0.42 b	1.18 ± 0.04 b
Mixture with Mustard	7.00 ± 0.14 b	432.1 ± 2.0 a	35.99 ± 0.98 a	1.84 ± 0.03 a
Three-species mixture	6.62 ± 0.21 b	436.0 ± 1.1 a	31.28 ± 0.68 b	0.84 ± 0.09 c
Anova test	***	***	*	***
Mustard				
Monoculture	0.83 ± 0.03 b	375.2 ± 5.51 b	9.01 ± 0.10 a	1.7 ± 0.12 a
Mixture with Oat	0.59 ± 0.03 c	386.5 ± 4.70 ab	7.22 ± 0.26 b	1.58 ± 0.11 ab
Mixture with Pea	1.51 ± 0.14 a	395.6 ± 7.7 ab	10.23 ± 0.63 a	1.21 ± 0.07 b
Three-species mixture	1.30 ± 0.09 a	397.7 ± 3.27 a	9.59 ± 0.40 a	1.55 ± 0.11 ab
Anova test	***	*	***	*

mixture with pea whereas its P content was highest in monoculture than in mixture.

Discussion

Importance of key species for biomass production

In our pot experiment, none of the species mixtures produced significantly more biomass than the respective monocultures. Mixing plant species with optimized ratios was thus not sufficient to improve the biomass production of the cover crops compared to monocultures, and this result contradicts our first hypothesis. However, our second hypothesis was verified because the presence of key species, such as pea and mustard, had strong impacts on biomass production. More precisely, the presence of pea had a significant positive effect on cover crop productivity, while mustard had a detrimental impact. Because of its ability to fix N from the atmosphere via bacterial symbiosis (Möller et al. 2008), pea allows high dry matter production under low N resources, as long as P and light conditions are favorable (Mengel 1994; Johnston 1995; Crews 1999). As a consequence, pea monoculture produced much more biomass than the two other monocultures, and all cover crop mixtures including pea kept this advantage, which is in accordance with the results from previous studies (Spehn et al. 2002; Temperton et al. 2007; Marquard et al. 2009). As expected, the presence of mustard induced a reduction in plant biomass production, which could be explained by a negative allelopathic effect on the other crop species (Al-Sherif et al. 2013). Overall, our findings suggest that species selection (i.e., presence of pea) rather than resource-use complementarity (i.e., mixtures of two or three crop species) drive cover crop productivity. However, even though complementarity effects were not observed in our study, it is not excluded that such effects might occur in mixtures with other or more cover crop species with different functional traits, as well as for other cover crop goals (i.e., improving soil structure or resistance to drought).

Oat benefits more from nutrients transfer from legumes than mustard

In contrast to our first hypothesis, we did not observe complementarity effects in cover crop mixtures to increase N and P concentrations in plant tissues compared to monocultures. However, the species-specific effects stated in our second hypothesis were confirmed because the presence of pea was the main driver of N and P concentrations in plant tissues. Therefore, it is not the mixture of species that improved nutrients absorption but the presence of key functional groups (Tilman et al. 1997; Huston et al. 2000; Spehn et al. 2002). Interestingly, the presence of pea increased nutrient concentrations in oat (Table 4). These observations were also made for N (Temperton et al. 2007; Möller et al. 2008; Nyfeler et al. 2011) and P acquisition by neighboring plants (Li et al. 2007; Hinsinger et al. 2011). As pea can fix N directly from the atmosphere, a reduced competition for soil nitrate could benefit oat in presence of pea. Furthermore, N transfer from pea to another species could occur because of additional available N released through degradation of highly N concentrated young root tissues and nodules (Dubach and Russelle 1994) and root exudates. It is likely that oat benefited from this improved soil N availability to take up more N, which increased the concentration of N in its root system. As a consequence, oat root system has a higher cation exchange capacity (Mclean et al. 1956; Grunes 1959), and is able to produce more phosphatase (Treseder and Vitousek 2001; Marklein and Houlton 2012) to absorb more P. Overall, the positive effect of pea on nutrient concentration in plant tissue was higher for oat than for mustard and this could be explained by differences in their root system architecture. Wide, fibrous root system of oat can explore the soil more efficiently, thus better exploiting extra-N and P induced by the presence of pea (Gallet et al. 2003; Richardson et al. 2009; Hinsinger et al. 2011). Oat also has a higher exchange surface with legume root system compared to the tap root of *Brassicaceae* and thus, it receives higher amount of N through facilitating processes (Pirhofer-Walzl et al. 2012). Furthermore, it was shown in competition experiments that grass root systems would be first to access the available P thanks to their high specific root length and root density (Caradus 1980; Richardson et al. 2009). On the agronomical point of view, we observed on average 6.4 times more N and 2.3 times more P stored in plant tissues in the cover crop treatments including pea, compared to those without pea (calculated from the data shown in Table 2). Among the seven crop treatments tested in our experiment, the monoculture of pea was also the most efficient in storing nutrients in plant tissues (both shoot and root).

Decomposition ability of cover crop biomass

The PCA biplot allowed to differentiate cover crops with pea (Fig. 1), which had higher N and P concentrations as well as lower C:N ratios, compared to those without pea. High nutrient content and low C:N ratio are crucial for a fast organic matter decomposition (Enríquez et al. 1993) and an efficient nutrient release to the following crop (Seneviratne 2000; Hobbie 2015). Looking at species level, oat in the three-species mixture and pea in all treatments produced high-quality biomass as indicated by their N concentrations above 15 mg N per g DM, which is the threshold for promoting organic N mineralization (Seneviratne 2000). With respect to P, oat biomass in the three-species mixture contains more than 2 mg P per g DM, which is the threshold for promoting P mineralization (Floate 1970; Murungu et al. 2011). All the treatments including pea showed a C:N ratio ranging from 12 to 18, allowing additional support to a fast mineralization of fresh organic matter for these cover crops. In contrast, all the treatments without pea had higher C:N ratios, with values ranging from 41 to 58, suggesting the production of a more recalcitrant organic matter (Enríquez et al. 1993). According to Liu and Sun (2013), a C:N ratio between 15 and 20 leads to N mineralization, while higher ratios induce N immobilization due to N consumption by microbes that degrade more recalcitrant organic matter. However, in pea monoculture, C:N ratio lower than 15 could lead to nitrate leaching or gaseous loss (Baggs et al. 2000).

Plant-soil-microbe interactions

Concerning the plant-microbe interactions, we did not observe higher microbial biomass nor higher microbial N and P concentrations in mixtures compared to monoculture as it was expected in our first hypothesis. The presence of key functional plant species had much stronger effects on these three variables, but our second hypothesis was also not verified. Indeed, we expected oat to provide favorable conditions to promote microbial biomass but, on the opposite, pea and mustard were the species with higher impact on microbial biomass and nutrient content.

Despite increasing soil N availability, pea exhibited a strong negative effect on microbial N, which can be explained by few mechanisms. First, the presence of legumes is known to stimulate nitrifying bacteria activity (Oelmann et al. 2007; Roux et al. 2013; Stephan et al. 2000), thus promoting N mineralization rather than immobilization in microbes. Higher microbial activity in the presence of pea is well supported by the observed increase in soil respiration (+104%) and absence of microbial biomass changes in pots that included pea. Second, it is likely that larger root system and higher root N uptake of pea induced stronger competition with microbes for soil N (Hodge et al. 2000). Third, as shown by our results, pea increased soil N in the form of NO_3^-, for which plants compete more strongly than microbes (Schimel et al. 1989; Dijkstra et al. 2012).

Soil available P decreased in presence of pea and this can be explained by the impact of pea on cover crop N uptake, as well as on P availability for other plants. Indeed, while increasing N uptake of the cover crop, the presence of pea also promoted P uptake in order to maintain stable plant N:P stoichiometry. Furthermore, pea is known to increase phosphorus availability for other plants, due to the release of organic anions or acids (Nuruzzaman et al. 2005; Hinsinger et al. 2011). As such, more nutrients being stored in plant biomass means less remaining in the soil. These results are also highlighted in the PCA (Fig. 1) where soil P is negatively correlated with plant N and P. In contrast to our second hypothesis, the presence of mustard did not increase soil P but induced a strong increase in microbial N (+36%) and a slight increase in microbial P. *Brassica* species are well known to release a large number and amount of allelopathic compounds into the soil as rhizodeposits (Gfeller et al. 2018; Al-Sherif et al. 2013). While certain phenolic compounds can catalyze the release of P, thus increasing soil P (Jones et al. 2009), many other root exudates produced by Brassicaceae, such as amino acids and organic acid-complexed P, can directly feed microbes and increase microbial N and P without modifying soil nutrient content (Hunter et al. 2014). We did not measure root exudates in our experiment but since no changes in soil N and P were observed, root exudation is likely the underlying mechanisms explaining the increase in microbial N and P in presence of mustard.

In our pot experiment, growth conditions were optimal for plant growth and did not exactly reproduce field conditions, thus yielding higher biomass production and nutrient sequestration than those often observed in field trials. However, since growth conditions were similar

for all seven cover crop treatments, our experiment still allows for comparison among treatments regarding the relative efficiency of each treatment to produce biomass and store nutrients. Furthermore, our results highlighted two important mechanisms by which particular cover crop species can improve soil nutrient availability and nutrient sequestration: one is adding and storing nutrient in plant biomass (e.g., pea) and the other is promoting microbial storage (e.g., mustard), both preventing nutrient leaching between main crops cultivation in agricultural soils.

Conclusion

Our findings highlight stronger selection effect (i.e., presence of key species) rather than complementarity effects (i.e., species mixtures) in increasing nutrient storage and biomass production in cover crops. More particularly, our results emphasize the needs to include legumes in cover crops for high N and P storage, particularly in nutrient-limited agricultural soils and when no fertilizer is applied. However, regarding the C:N ratio of the cover crop biomass, pea monoculture is not ideal because the degradation of its litter with an excess of N might lead to gaseous N loss. This is why, among the seven cover crops tested in our experiment, the oat-pea mixture and the three-species mixture are those recommended since they both allow high biomass production while complying with most of the desired cover crop functions. Furthermore, they have a high N and P storage capacity in plant tissues, with C:N ratio close to 15 leading to a quick degradation of the litter, ensuring both mineralization and microbial storage. Furthermore, these mixtures increased soil nutrient availability and microbial C and P content compared to the other cover crop treatments. The oat-pea mixture produced about 12% more plant biomass than the three-species mixture and this is a major advantage when targeting high litter biomass production in cover crops. On the other hand, the three-species mixture stored 60% more N in the microbial pool, and should be favored when aiming to reduce N leaching.

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