Catch crop mixtures have higher potential for nutrient carry-over than pure stands under changing environments

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ABSTRACT

Winter catch crops are grown to scavenge nutrients over a period of unfavorable growth conditions and to conserve nutrients for subsequent release to the following main crop. Since environmental conditions have a strong impact on the growth and nutrient capture in roots and shoots of individual catch crop species, we anticipated that mixtures will be more durable and efficient in nutrient capture due to compensatory effects among component species. We tested this hypothesis and determined the nitrogen and phosphorus accumulation in the shoots and roots of four catch crop species grown in pure vs. mixed stands at two sites for two or three years. Element concentrations were determined in the root and shoot biomass of each species and used to calculate the nutrient pool fixed in the root or shoot biomass. A qPCR-based technique was applied to quantify the root biomass of individual species based on species-specific DNA sequences. Despite considerable variation across environments, the overall plant biomass of white mustard (*Sinapis alba*), lacy phacelia (*Phacelia tanacetifolia*) and bristle oat (*Avena strigosa*) was similar and higher than that of Egyptian clover (*Trifolium alexandrinum*). While pure stands varied 6- to 24-fold in shoot biomass depending on environmental conditions, the variation was only ~3-fold for catch crop mixtures, with less pronounced variation in the root biomass. In general, the root biomass was comparable to the shoot biomass in each species. Roots contributed 26–46% of the nitrogen and 36–48% of the phosphorus to the total accumulation of these nutrients in the catch crop biomass, thus emphasizing the importance of plant roots as belowground nutrient pool for potential carry-over of nutrients to the subsequent crop. Although the mixture was mostly dominated by two of the four species, namely mustard and phacelia, it captured similar or even larger amounts of nutrients than the best-performing pure stand under any growth condition. This was the case for shoot- and for root-bound nutrients. Our results indicate that catch crop mixtures have higher durability than pure cultures to environmental variations. The amount of nitrogen captured by the mixture meets the average postharvest nitrogen that is left over by a wide range of cash crops, thus emphasizing that catch crop mixtures represent an efficient nutrient management tool in crop rotations.

1. Introduction

In crop rotations, winter catch crops are grown to scavenge soil nutrients over winter and carry them over to the subsequently grown main crop (Sieling, 2019). Nutrient capture is of particular importance for the nitrogen (N) form nitrate, which is prone to leaching into deeper soil layers (Jensen, 2006). Once below the root zone, nitrate transfer into the hydrosphere can cause severe environmental problems, such as

eutrophication or hypoxia (Galloway and Cowling, 2002). Sieling (2019) reviewed that catch crops can decrease leaching by up to 100 kg N ha^{-1} depending on the species, catch crop management and geographical location. Considering the beneficial traits of catch crop species, the efficiency of uptake and accumulation of the soil mineral N forms nitrate and ammonium is mostly associated with shoot biomass formation, i.e. carbon and N accumulation of a catch crop (Kramberger et al., 2013; Wendling et al., 2016), and the development of a deep root system (Thorup-Kristensen, 2001, 2006; Heuermann et al., 2019).

In addition to scavenging nitrate and other readily leachable nutrients, catch crops may also improve the availability of less soluble nutrients, such as phosphorus (P). The excessive use of P-containing organic and mineral fertilizers in some areas is problematic in terms of eutrophication potential and finite global P reserves (Cordell et al., 2009). Thus, the cycling of natural P reserves from organic and inorganic P pools in agricultural soils must be improved. Catch crop species may contribute to P mobilization from sparingly soluble precipitates, incorporate P into their biomass and release it in plant-available forms to the following crop during the mineralization of their biomass (Lambers et al., 2006; Eichler-Löbermann et al., 2008). The release of protons, organic acids or P-mobilizing enzymes, such as phytases, symbiosis with mycorrhizae and a large number of lateral roots and root hairs are beneficial for the efficient acquisition of P (Dakora and Phillips, 2002; Lambers et al., 2006; Lynch, 2019). In particular, species classified as shallow rooting, such as phacelia, ryegrass or buckwheat (Kutschera et al., 2009), have been described as the most efficient for P acquisition (Eichler-Löbermann et al., 2008).

Individual plant species tend to form root systems with either steep or shallow root angles that are beneficial for nutrient acquisition from either deep soil layers or the topsoil, respectively, while mixtures of catch crop species may overcome such trade-offs and improve nutrient management by accessing a larger soil volume. Compared to pure stands, catch crop mixtures can be superior in aboveground biomass formation and related nutrient fixation due to their higher durability under unfavorable conditions (Wortman et al., 2012; Kramberger et al., 2013; Elhakeem et al., 2019; Khan and McVay, 2019). This advantage can be based on the stimulation of microbial biomass via enhanced carbon delivery to the rhizosphere (Gentsch et al., 2020), the release of specific root exudates promoting beneficial microbial taxa (Yu et al., 2021), the beneficial effects of root microbiomes on improving root growth of component species (Mommer et al., 2010), or the exudate-based transfer of nutrients between species (Paynel and Cliquet, 2003). In addition, functional complementarity within mixtures relying on vertical root niche differentiation may result in more efficient exploitation of the rooted soil volume. In fact, studies have reported greater belowground biomass yield for plant communities with diverse species compared with communities with fewer species (Reich et al., 2004; Mommer et al., 2010; Heuermann et al., 2019).

To date, nutrient capture by catch crops has been evaluated almost exclusively in the shoot biomass, as in the studies of Eichler-Löbermann et al. (2008), Sullivan and Andrews (2012), Zaniewicz-Bajkowska et al. (2013), Koefender et al. (2016) and Xavier et al. (2017). However, estimating the contribution of nutrient capture in the root biomass is implicitly necessary to exploit and estimate the full potential of catch crops for efficient nutrient carry-over in sustainable crop rotations (Hupe et al., 2016). By determining biomass and nutrient concentrations in roots washed out from excavated soil (Kuo et al., 1997; Redin et al., 2018), soil cores (Wendling et al., 2016) or ingrowth cores (Komainda et al., 2016), previous studies have estimated the share of roots in total nutrient capture by catch crops up to 20–50 cm soil depth. However, root washing methods risk overestimating root biomass whenever the co-collection of soil organic matter, whose origin derives from sources other than the actual grown plant species, cannot be avoided (Hirte et al., 2017). DNA-based root quantification methods can overcome this problem and can even assign root biomass to different species in mixtures (Haling et al., 2011; Huang et al., 2013; Heuermann et al., 2019).

The current study focused on the nutrient scavenging potential of the root biomass of catch crop species and the nutrient ratio fixed in the belowground versus aboveground biomass of catch crops cultivated in pure or mixed stands. We presumed that in changing environments a mixture with higher biodiversity will increase the robustness of nutrient binding not only in the shoot (Khan and McVay, 2019) but also in the root biomass. First, we compared the biomass and N and P concentrations in four different species reported to differ in root morphology, i.e., white mustard (*Sinapis alba*), lacy phacelia (*Phacelia tanacetifolia*), bristle oat (*Avena strigosa*) and Egyptian clover (*Trifolium alexandrinum*; Kutschera et al., 2009). The root biomass was determined at different soil depths using a DNA-based quantification method. We then addressed whether a mixture of these species shows a larger potential for nutrient scavenging in the root biomass than pure stands, especially under variable growth conditions. We therefore repeated a field experiment in five test environments and grew the four species either in pure stands or in a four-species mixture for subsequent determination of the biomass or N and P accumulation in the shoots and roots.

2. Materials and methods

2.1. Plant species and growth conditions

Experiments were conducted at two sites in Germany: Asendorf in Lower Saxony, 52°45′48.4″N 9°01′24.3″E, and Triesdorf in Bavaria, 49°12′36.5″N 10°38′33.9″E. The soils were classified as Stagnic Cambisols (WRB, 2015). In Asendorf, the uniformly textured soil was a silty loam, while in Triesdorf the substrate was more heterogeneous and ranged from sandy loam to sandy clay-loam.

Four catch crop species, namely, white mustard cv. Litember, lacy phacelia cv. Bee Happy, bristle oat cv. Panache and Egyptian clover cv. Alex were grown either in pure stands or in a mixture of the four species in a randomized complete block design that included plots of 7.3 m × 6 m in three replicates. In Asendorf, the catch crop stands were established in 2015, 2016 and 2017; in Triesdorf, the catch crops were grown in 2016 and 2017. They were sown on 3 September 2015, 20 August 2016 and 15 August 2017 in Asendorf and on 24 August 2016 and 15 August 2017 in Triesdorf. Due to the late sowing date in Asendorf in 2015, the seedlings were covered for 12 days by a fiber mat, which improved seedling development under glasshouse-like conditions. Winter wheat (*Triticum aestivum*) cv. Patras was the preceding crop in every environment. In Asendorf, plants were fertilized with liquid urea ammonium nitrate at a dose of 50 kg N ha^{-1} on 7 September 2015 and of 40 kg N ha^{-1} in the two following years (7 September 2016 and 28 August 2017). In Triesdorf, catch crops received 27 or 30 kg N ha^{-1}, as calcium ammonium nitrate, on 29 September 2016 or 8 September 2017, respectively.

Table 1

Monthly average temperature and precipitation during the catch crop cultivation period at site Asendorf in 2015, 2016 and 2017 and at site Triesdorf in 2016 and 2017 as recorded by local weather stations. Note: For the sake of data comparability, Table 1 also includes weather recordings from 2015 and 2016 taken from Heuermann et al. (2019).

	Asendorf			Triesdorf	
	2015	2016	2017	2016	2017
Avg. Temperature [°C]					
Aug	19.2	17.6	17.1	17.8	17.9
Sep	13.4	17.6	13.5	16.1	11.7
Oct	8.9	9.1	12.1	7.5	10.0
Nov	8.4	4.2	6.1	3.0	3.8
Ø 4-month	12.5	12.1	12.2	11.1	10.9
Precipitation [mm]					
Aug	135.0	20.6	99.4	38.0	66.8
Sep	55.4	29.6	165.2	39.1	55.2
Oct	63.2	29.4	129.2	54.8	63.4
Nov	175.4	66.4	88.4	52.5	60.4
Σ 4-month	429.0	146.0	482.2	429.0	245.8

Weather data for the seasons are shown in Table 1.

At both sites, the following seeding rates [seeds m^{-2}] were applied: Mustard (pure stand) – 300, phacelia (pure stand) – 706, oat (pure stand) – 588, clover (pure stand) – 833, mustard (mixture) – 67, phacelia (mixture) – 294, oat (mixture) – 53, and clover (mixture) – 233. By choosing these seeding rates, we targeted for similar overall shoot biomass in pure and mixed stands, balanced the differences in speed of juvenile development among the species and considered the competitiveness of individual species based on long-term experience of the seed manufacturer Deutsche Saatveredelung (Lippstadt, Germany). Further information on the field management and on soil properties are given in Heuermann et al. (2019).

2.2. Sampling and processing of plant material and soil cores

For determination of N and P in the plant material of each species, we cut five individual shoots and manually pulled out two to five individual roots per species from each plot when day temperatures started to decrease below 5 °C in autumn (Asendorf: 27 October 2015 / 9 November 2016 / 14 November 2017, Triesdorf: 2 November 2016 / 9 November 2017). Soil adhering to roots was removed by rinsing with tap water before roots were pooled. The shoots were kept separately. All plant parts were dried at 60 °C to constant weight. The dry shoot and root samples were ground in a swing mill (RS200, Retsch, Haan, Germany).

For biomass determination per unit area, three microplots per plot, which were 100 × 100 cm in Asendorf and Triesdorf in 2017 and 50 × 50 cm in Triesdorf in 2016, were used to cut all plants just above the soil surface. Shoots were sorted by species and dried separately at 60 °C to constant weight. The root dry mass was determined in soil cores using a qPCR-based method as described in Heuermann et al. (2019). In brief, we first developed species-specific primers that amplified parts of the internal transcribed spacer region 1 or 2 to discriminate the individual catch crops against other species, pregrown wheat and *Spatiphyllum* (natively occurring in South America but employed here as internal control). Then, we grew individual catch crops in meshes in their intact catch crop stands in the field and washed their roots carefully after approximately eight weeks. The roots were frozen and ground, and distinct amounts in the range from 10 to 150 mg of fresh root material were used to extract genomic DNA. Additional aliquots were taken to determine the corresponding dry weights (DWs). After quantitative PCR using the respective species-specific primers, we set up standard curves for individual catch crops plotting cycle threshold values against log (root DW). In the field, we took soil cores (6 cm diameter) down to a soil depth of 70 cm and cut them into 4-cm slices around 0, 10, 20, 30, 40, 50, 60 and 70 cm. We washed the soil samples through sieves with a 0.4 mm mesh size to reduce the soil:root ratio, which yielded higher gDNA quantity and quality. The samples were frozen and ground, and ~600 mg DW was used for gDNA extraction. Additionally, 20 mg of *Spatiphyllum* leaf material was added to every sample, which served as an internal control for gDNA extraction efficiency against the soil matrix remaining after washing. In the following quantitative PCR assays with species-specific primers, we determined the cycle threshold values for every catch crop DNA as well as for *Spatiphyllum* and calculated the root DW at different soil depths based on the respective standard curves. The same procedure was applied to fallow plots to correct for artifacts from field-internal DNA stocks of previous catch crop species.

2.3. Elemental analysis of plant material

Nitrogen concentrations were determined in 1.5 mg ground plant material using an elemental analyzer (EuroEA3000, Hekatech, Wegberg, Germany). Further elements were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES; iCAP 7400 duo, Thermo Fischer Scientific, Dreieich, Germany) as described in Eggert and von Wirén (2013). From each plot, five individual shoot samples and one root sample pooled from two to five individual plants were analyzed for every species (see 2.2). The mean of the five shoot samples was used as the element concentration value for that specific plot. Total element accumulation in plant biomass per area was calculated as the product of the mean shoot biomass in three microplots or the mean root biomass upscaled from three soil cores per plot (see Section 2.2) and the mean shoot or root element concentrations. Data from 2015 and 2016 were recalculated from Heuermann et al. (2019), although due to sample loss in 2015 and 2016, only one pooled root sample per species was analyzed to determine element concentrations. In Table 3, the root element concentrations for 2015 and 2016 are represented by a single value that is identical for individual species in pure and mixed stands. This resulted in only one root value being used in further analyses: Root:shoot ratios in Table 3 as well as total element accumulation in the roots in Fig. 2 and Fig. 3 in 2015 and 2016 are based on only one root element concentration value obtained from three plots (n = 3).

2.4. Analysis of nutrient stocks in soil samples

Soil samples were extracted in three replicates per plot using a manual soil corer being 2 cm in diameter in increments of 0–30, 30–60, and 60–90 cm depth. Then, one pooled sample per depth was created and transported in a cooler to the laboratory for the fresh extraction of soil mineral N (N$_{min}$). For further analysis, the samples were dried at 40 °C and sieved through 2 mm-sized meshes if necessary. Samples for determining bulk density were collected nondestructively using a stainless core cutter (100 cm^3), and bulk density was determined gravimetrically after drying at 105 °C. N$_{min}$ was extracted as nitrate and ammonium with a 12.5 mM CaCl$_2$ solution and measured by an autoanalyzer (SAN-plus, Skalar Analytical B.V., Breda, The Netherlands).

Plant-available P was measured according to Ziadi and Tran (2006). In brief, dry soil was extracted with Mehlich 3 at a 1:10 (w/v) soil:solution ratio and filtered. Mehlich 3-extractable P (P$_{ex}$) was determined in extracts by ICP-OES (Varian 725-ES, Palo Alto, California). Data for N$_{min}$ and P$_{ex}$ stocks in the soil before catch crop cultivation are given in Table 2.

2.5. Statistical analysis

First, the mean of all replicated data for biomass and for N and P concentration from one plot was calculated; in mixture-plots, this was done per individual species. Then, mean biomasses were multiplied with mean respective element concentrations yielding element accumulation per plot. In mixture-plots, we calculated a sum of biomass, N or P accumulation of the individual component species.

Statistical analyses were undertaken in R version 4.1.0 (R Core Team, 2021).

In each environment, differences among the five catch crop variants (pure mustard, pure phacelia, pure oat, pure clover, sum of species in the mixture) in biomass, N and P accumulation were calculated based

Table 2

Soil stocks of mineral nitrogen (N$_{min}$) and Mehlich 3-extractable phosphorus (P$_{ex}$) in the 0–90 cm soil depth before the catch crop cultivation periods in Asendorf in 2015, 2016 and 2017 and in Triesdorf in 2016 and 2017. Values are means ± SD; n = 19–21.

	N$_{min}$ [kg ha^{-1}]		P$_{ex}$ [kg ha^{-1}]	
	Asendorf	Triesdorf	Asendorf	Triesdorf
Sampling date				
2015	31.7.2015		31.7.2015	
2016	3.8.2016	16.8.2016	3.8.2016	16.8.2016
2017	9.8.2017	7.8.2017	9.8.2017	7.8.2017
N$_{min}$ / P$_{ex}$				
2015	52.1 ± 10.3		2155.4 ± 253.0	
2016	58.9 ± 13.6	12.2 ± 4.0	1019.6 ± 141.0	741.6 ± 212.4
2017	30.6 ± 4.2	23.9 ± 5.1	1300.8 ± 345.3	849.8 ± 235.8

Table 3

Concentrations of N and P in the shoot and root dry biomass and root:shoot ratios of respective element concentrations of four catch crop species cultivated in pure or mixed stands in five environments. Mustard, phacelia, oat and clover were cultivated either alone or in a mixture of all four species in 2015, 2016 and 2017 in Asendorf and in 2016 and 2017 in Triesdorf. Table shows means ± SD; n = 3 [shoots, roots in 2017], n = 1 [roots in 2015 and 2016], n.a.=not analyzed. In pure stands, estimated means are extracted from model 1 and when followed by a common capital letter not significantly different at the 5% significance level; in mixtures, estimated means are extracted from model 2 and when followed by a common small letter not significantly different at the 5% significance level; n.s.=not significant., n.t.=not tested. Estimated means from mixtures followed by an asterisk are significantly different at the 5% significance level to the respective species in pure stands according to model 3. The root:shoot ratio shows the mean root-to-shoot concentration ratio for N or P; n = 3 (Note: As in 2015 and 2016 only one root N value was available, statistical evaluation is omitted).

	Shoot concentration [mg g⁻¹ DW]		Root concentration [mg g⁻¹ DW]		Root:Shoot ratio	
	N	P	N	P	N	P
Asendorf 2015						
Pure stand						
Mustard	45.5 ± 6.6 [n.s.]	7.0 ± 0.9 [B]	14.9 [n.t.]	n.a.	0.3 ± 0.05 [C]	n.a.
Phacelia	40.7 ± 1.1	9.2 ± 0.7 [A]	27.4	n.a.	0.7 ± 0.02 [A]	n.a.
Oat	37.5 ± 1.3	9.0 ± 0.5 [A]	19.8	n.a.	0.5 ± 0.02 [B]	n.a.
Clover	43.2 ± 7.4	6.6 ± 0.8 [B]	30.2	n.a.	0.7 ± 0.11 [A]	n.a.
Mixed stand						
Mustard	40.1 ± 7.5 [n.s.]	6.9 ± 0.3 [d]	14.9 [n.t.]	n.a.	0.4 ± 0.07 [c]	n.a.
Phacelia	46.6 ± 6.6	14.0 ± 2.4 [a]	27.4	n.a.	0.6 ± 0.08 [b]	n.a.
Oat	43.6 ± 0.6 *	10.6 ± 1.0 [bc] *	19.8	n.a.	0.5 ± 0.01 [c]	n.a.
Clover	42.7 ± 4.7	8.2 ± 0.8 [cd]	30.2	n.a.	0.7 ± 0.08 [a]	n.a.
Asendorf 2016						
Pure stand						
Mustard	27.7 ± 0.7 [AB]	2.5 ± 0.1 [B]	9.2 [n.t.]	3.4 [n.t.]	0.3 ± 0.01 [B]	1.4 ± 0.07 [A]
Phacelia	24.1 ± 5.6 [B]	3.9 ± 0.4 [A]	8.2	2.5	0.4 ± 0.08 [B]	0.6 ± 0.06 [C]
Oat	21.9 ± 0.5 [B]	2.9 ± 0.1 [B]	5.7	1.3	0.3 ± 0.01 [B]	0.5 ± 0.02 [D]
Clover	33.4 ± 3.5 [A]	2.9 ± 0.4 [B]	27.2	3.2	0.8 ± 0.09 [A]	1.1 ± 0.14 [B]
Mixed stand						
Mustard	23.1 ± 10.5 [n.s.]	2.7 ± 0.1 [b]	9.2 [n.t.]	3.4 [n.t.]	0.4 ± 0.16 [b]	1.3 ± 0.06 [a]
Phacelia	28.7 ± 5.4 *	4.3 ± 1.0 [a]	8.2	2.5	0.3 ± 0.06 [bc]	0.6 ± 0.14 [c]
Oat	23.7 ± 1.2	3.4 ± 0.3 [ab]	5.7	1.3	0.2 ± 0.01 [c] *	0.4 ± 0.03 [d]
Clover	35.9 ± 1.3	3.4 ± 0.1 [ab]	27.2	3.2	0.8 ± 0.03 [a]	0.9 ± 0.03 [b]
Asendorf 2017						
Pure stand						
Mustard	15.3 ± 0.3 [B]	2.9 ± 0.3 [D]	7.7 ± 0.5 [B]	3.2 ± 0.6 [B]	0.5 ± 0.03 [B]	1.1 ± 0.11 [B]
Phacelia	15.2 ± 1.0 [B]	5.2 ± 0.1 [A]	6.1 ± 0.4 [B]	5.4 ± 0.6 [A]	0.4 ± 0.05 [B]	1.0 ± 0.09 [B]
Oat	16.9 ± 1.4 [B]	4.6 ± 0.3 [B]	7.3 ± 1.6 [B]	3.1 ± 1.0 [B]	0.4 ± 0.11 [B]	0.7 ± 0.21 [C]
Clover	32.1 ± 2.7 [A]	3.4 ± 0.1 [C]	33.9 ± 0.4 [A]	4.8 ± 0.7 [A]	1.1 ± 0.10 [A]	1.4 ± 0.16 [A]
Mixed stand						
Mustard	15.1 ± 1.8 [d]	3.2 ± 0.5 [c]	4.9 ± 0.5 [b] *	2.9 ± 0.7 [c]	0.3 ± 0.02 [b] *	0.9 ± 0.20 [b]
Phacelia	21.2 ± 2.0 [c] *	6.1 ± 0.9 [a]	8.4 ± 0.8 [b] *	6.8 ± 0.3 [a]	0.4 ± 0.01 [b]	1.1 ± 0.12 [b]
Oat	24.6 ± 1.3 [b] *	5.0 ± 0.5 [b]	7.8 ± 2.8 [b]	2.5 ± 0.7 [c]	0.3 ± 0.12 [b]	0.5 ± 0.12 [c]
Clover	34.0 ± 1.3 [a]	3.3 ± 0.3 [c]	33.3 ± 3.7 [a]	4.8 ± 0.7 [b]	1.0 ± 0.13 [a]	1.4 ± 0.15 [a]
Triesdorf 2016						
Pure stand						
Mustard	31.4 ± 0.4 [C]	4.3 ± 0.5 [B]	18.3 [n.t.]	2.8 [n.t.]	0.6 ± 0.01 [B]	0.7 ± 0.08 [A]
Phacelia	32.2 ± 3.4 [C]	5.9 ± 0.8 [AB]	17.3	4.9	0.5 ± 0.06 [BC]	0.8 ± 0.11 [A]
Oat	49.1 ± 4.7 [A]	7.0 ± 0.6 [A]	21.9	2.3	0.4 ± 0.05 [C]	0.3 ± 0.03 [B]
Clover	39.5 ± 2.3 [B]	4.8 ± 0.8 [B]	27.7	4.0	0.7 ± 0.04 [A]	0.8 ± 0.12 [A]
Mixed stand						
Mustard	31.2 ± 0.8 [c]	4.6 ± 0.3 [c]	18.3 [n.t.]	2.8 [n.t.]	0.6 ± 0.01 [b]	0.6 ± 0.04 [b]
Phacelia	37.2 ± 2.2 [b]	6.5 ± 0.6 [a]	17.3	4.9	0.5 ± 0.03 [c]	0.8 ± 0.07 [a]
Oat	36.5 ± 2.5 [b]	6.2 ± 0.3 [a]	21.9	2.3	0.6 ± 0.04 [b] *	0.4 ± 0.02 [c]
Clover	41.1 ± 0.0 [a]	4.9 ± 0.4 [b]	27.7	4.0	0.7 ± 0.003 [a]	0.8 ± 0.06 [a]
Triesdorf 2017						
Pure stand						
Mustard	12.9 ± 0.6 [B]	3.2 ± 0.4 [n.s.]	5.6 ± 0.1 [AB]	3.4 ± 0.1 [AB]	0.4 ± 0.03 [B]	1.0 ± 0.11 [A]
Phacelia	12.7 ± 0.3 [B]	4.6 ± 0.4	5.3 ± 0.6 [B]	4.3 ± 0.1 [AB]	0.4 ± 0.04 [B]	0.9 ± 0.09 [AB]
Oat	13.9 ± 1.6 [B]	4.3 ± 0.9	3.6 ± 0.7 [B]	2.5 ± 1.3 [B]	0.3 ± 0.02 [C]	0.6 ± 0.27 [B]
Clover	30.4 ± 0.5 [A]	4.5 ± 0.5	27.7 ± 1.8 [A]	5.4 ± 1.1 [A]	0.9 ± 0.04 [A]	1.2 ± 0.14 [A]
Mixed stand						
Mustard	16.3 ± 0.7 [c] *	3.4 ± 0.2 [b]	6.8 ± 0.04 [b] *	3.9 ± 0.1 [a] *	0.4 ± 0.02 [b]	1.2 ± 0.07 [a]
Phacelia	15.3 ± 1.8 [c]	5.3 ± 0.9 [a]	4.8 ± 0.03 [c]	4.3 ± 0.1 [a]	0.3 ± 0.04 [c]	0.8 ± 0.13 [b]
Oat	22.1 ± 0.8 [b] *	5.8 ± 0.5 [a]	5.6 ± 1.2 [bc] *	2.6 ± 0.3 [b]	0.3 ± 0.06 [c]	0.4 ± 0.05 [c]
Clover	28.1 ± 1.6 [a]	4.0 ± 0.1 [b]	22.3 ± 0.9 [a] *	4.6 ± 0.8 [a]	0.8 ± 0.01 [a] *	1.2 ± 0.21 [a]

Table 3 (*continued*)

on the following linear mixed model with the lme4 package version 1.1.27.1 (Bates et al., 2015):

$$Y_{ij} = \mu + cv_i + b_j + e_{ij} \qquad \text{(model 1)}$$

Y_{ij}… biomass, N or P accumulation of the ith catch crop variant in the jth block; μ… intercept term ($\mu = 0$); cv_i… effect of the ith catch crop variant treated as fixed effect; b_j… effect of the jth block treated as random as effect; e_{ij}…residual errors.

For N and P concentrations, we calculated differences among pure-cultured species according to model 1 after sub setting the dataset for the cultivation form "pure". In order to evaluate differences among species cultivated in the mixture, we sub set the data by the cultivation form "mixture". Since every block contained exactly one mixture-plot, we treated mixture-plot as random factor:

$$Y_{ij} = \mu + cs_i + m_j + e_{ij} \qquad \text{(model 2)}$$

Y_{ij}… N or P concentration of the ith catch crop species in the jth mixture-plot; μ… intercept term ($\mu = 0$); cs_i… effect of the ith catch crop species
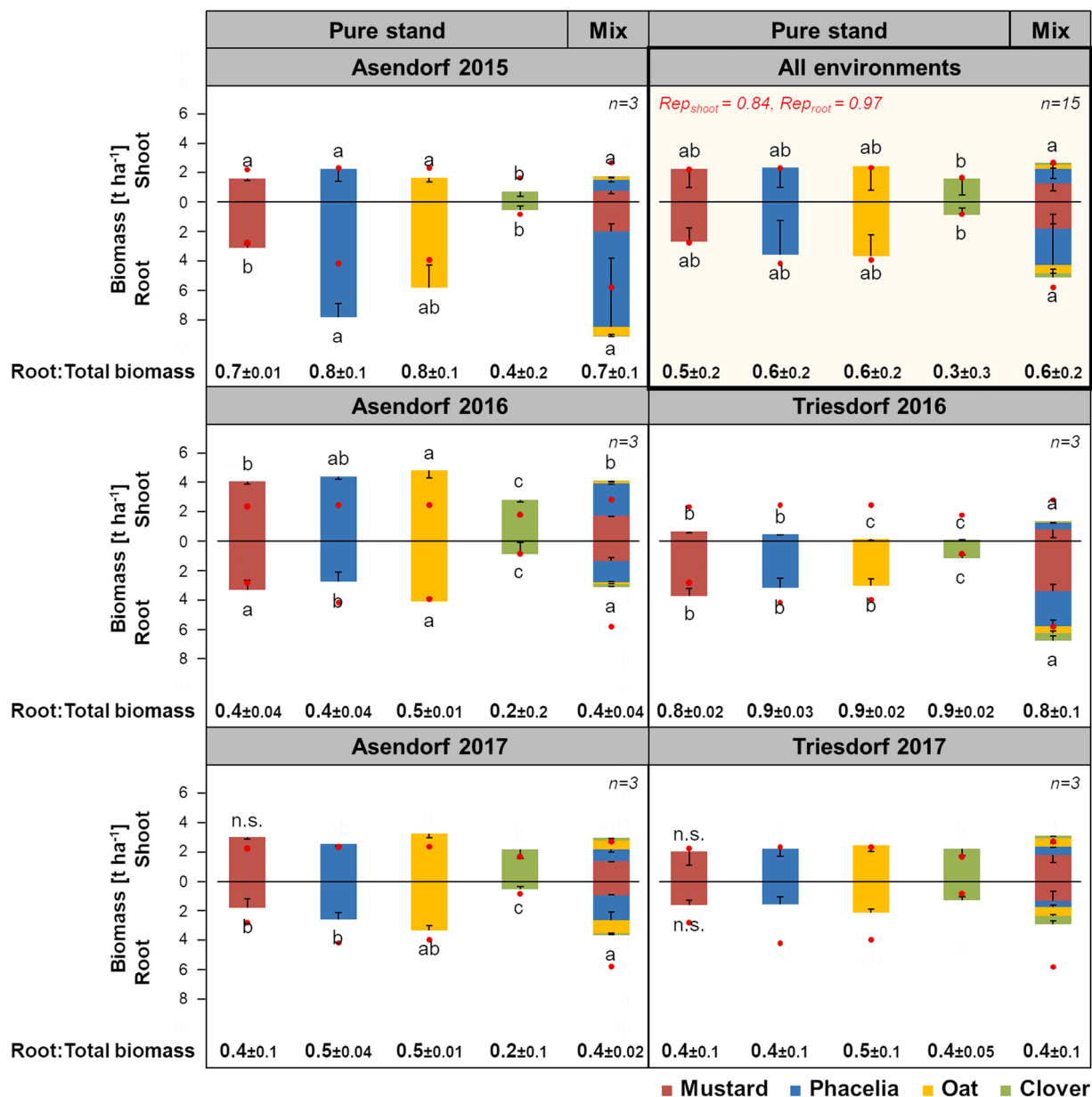
Fig. 1. Total shoot and root biomass of the four catch crop species and the mixture in five environments and across all environments. Mustard, phacelia, oat and clover were cultivated either alone or in a mixture ("Mix") of all four species in 2015, 2016 and 2017 in Asendorf and in 2016 and 2017 in Triesdorf. Bars (upward: shoot, downward: root) show the mean -SD; n = 3 or 15 plots. Red dots show BLUEs over all plots from all environments; n = 15. In individual environments, BLUEs are shown for comparison. In individual environments, estimated means are extracted from model 1 and when followed by a common letter not significantly different at the significance 5% level; across environments estimated means are extracted from model 4 and when followed by a common letter not significantly different at the 5% significance level; n.s.=not significant. "Root:Total biomass" ratio refers to root biomass to a soil depth of 70 cm relative to the total plant biomass; n = 3 or 15 plots. The adjusted repeatability for species ("Rep") is given in the upper right panel. For sake of comparability to data from 2017, biomass data of 2015 and 2016 are included from Heuermann et al. (2019).

treated as fixed effect; m_j... effect of the jth mixture-plot treated as random effect; e_{ij}...residual errors.

Differences in N or P concentration within one species between cultivation forms (="pure" or "mixture") were evaluated based on the following model after sub setting the datasets for the individual species:

$$Y_{ij} = \mu + c_i + b_j + e_{ij} \quad \text{(model 3)}$$

Y_{ij}... N or P concentration of respective catch crop species under the ith cultivation form in the jth block; μ... intercept term (μ = 0); c_i... effect of the ith cultivation form treated as fixed effect; b_j... effect of the jth block treated as random as effect; e_{ij}...residual errors.

In all cases, normality was checked based on the residuals with

diagnostic plots as described by Kozak and Piepho (2018) and log-transformation of the data was performed when necessary. The emmeans package version 1.7.0 (Lenth, 2021) was used to calculate significant differences between fixed effects based on the estimated means (method="pairwise", adjust="fdr", alpha=0.05).

Across all environments, differences among the five catch crop variants in biomass, N and P accumulation were calculated based on the following linear mixed model with the lme4 package:

$$Y_{ijkl} = \mu + cv_i + b_j + y_k + s_l + cvs_{il} + cvy_{ik} + ys_{kl} + by_{jk} + bs_{jl} + e_{ijkl}$$

$$\text{(model 4)}$$

Y_{ijkl}... biomass, N or P accumulation or root:shoot ratio of biomass, N or
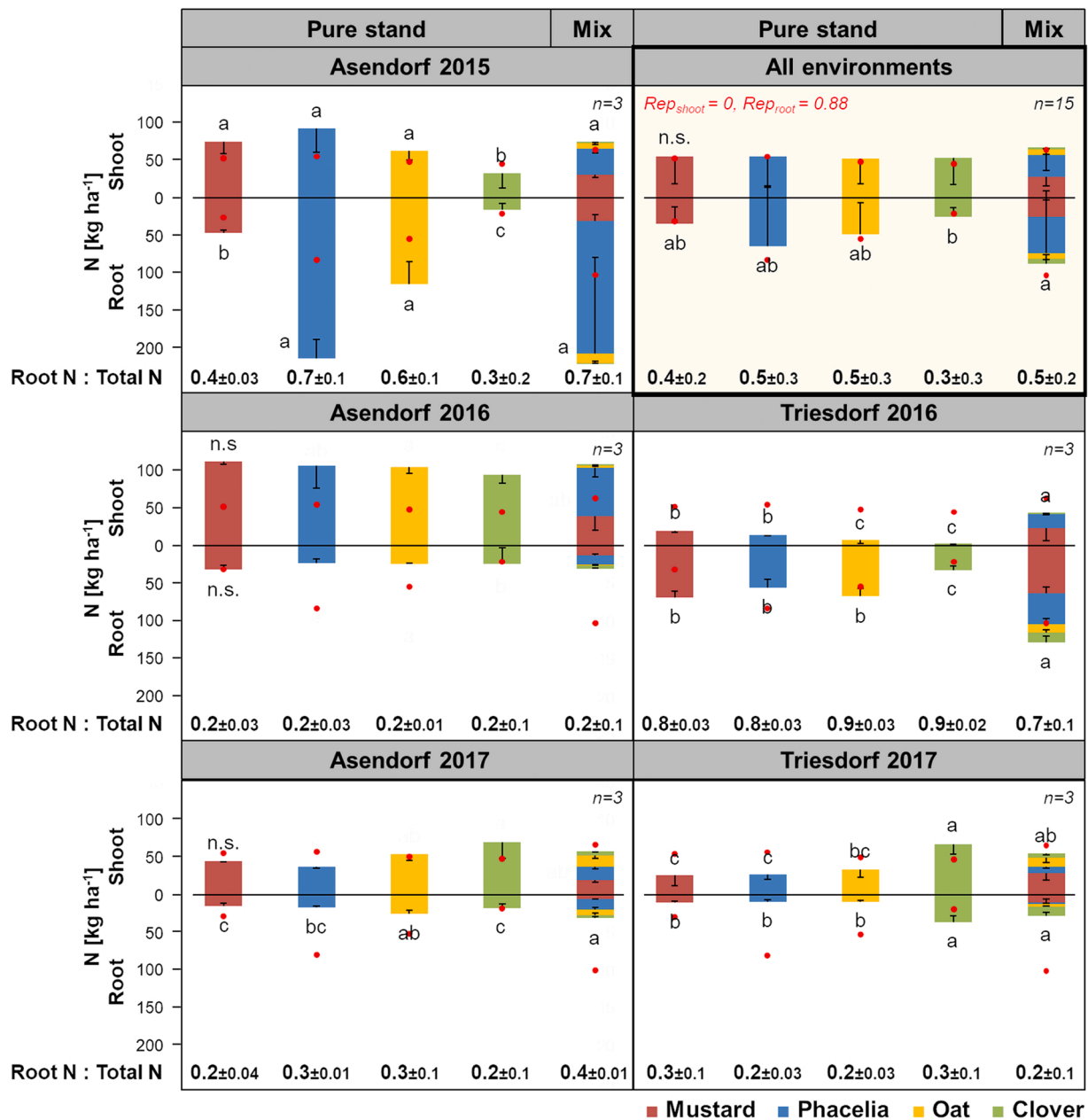
Fig. 2. Total N accumulation in the shoots and roots of the four catch crop species and the mixture in five environments and across all environments. Shoot and root N contents were determined in mustard, phacelia, oat and clover cultivated either alone or in a mixture ("Mix") of all four species in 2015, 2016 and 2017 in Asendorf and 2016 and 2017 in Triesdorf**.** Bars (upward: shoot, downward: root) show the mean -SD; n = 3 or 15 plots. Red dots show BLUEs across all environments; n = 15. In individual environments, BLUEs are shown for comparison. In individual environments, estimated means are extracted from model 1 and when followed by a common letter not significantly different at the significance 5% level; across environments estimated means are extracted from model 4 and when followed by a common letter not significantly different at the 5% significance level; n.s.=not significant. "Root N: Total N" shows the ratio of root-bound N to N bound in the total plant biomass; n = 3 or 15 plots. The adjusted repeatability for species ("Rep") is given in the upper right panel. Note: A Rep of 0 means, that no variance was explained by the species alone but by the interaction of species x year and species x location.

P accumulation of the ith catch crop variant in the jth block in the kth year at the lth site; μ... intercept term ($\mu = 1$); cv_i... effect of the ith catch crop variant treated as fixed effect;

b_j... effect of the jth block treated as random as effect; y_k... effect of the kth year treated as random effect;

s_l... effect of the lth site treated as random effect; cvs_{il}... interaction term of the ith effect of catch crop variant with the lth effect of site treated as random effect; cvy_{ik}... interaction term of the ith effect of catch crop variant with the kth effect of year treated as random effect; ys_{kl}... interaction term of the lth effect of site with the kth effect of year treated as random effect; by_{jk}... interaction term of the jth effect of block with the

k^{th} effect of year treated as random effect; bs_{jl}... interaction term of the jth effect of block with the lth effect of site treated as random effect; e_{ijkl}... residual errors.

Only for shoot N accumulation this model did not converge. Thus, we iteratively removed b_j and bs_{jl} as those effects did not significantly improve the Akaike information criterion of the model fit. Then, the model converged. Normality was checked based on residuals as described above. By treating catch crop species as fixed effect, differences in biomass, N and P accumulation among the catch crop variants were calculated based on estimated means as described before, and best linear unbiased estimators (BLUEs) were extracted with the lme4
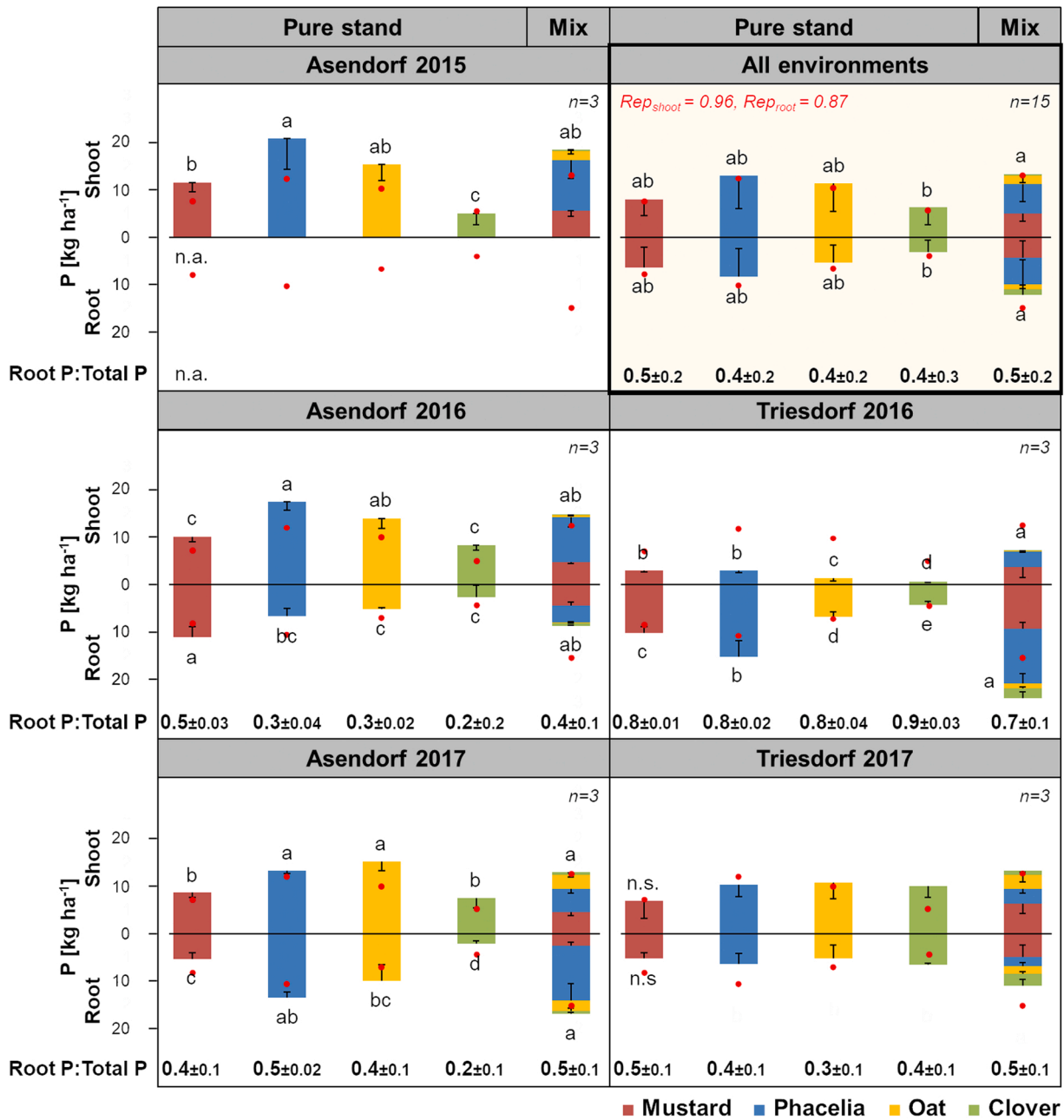
Fig. 3. Total P accumulation in the shoots and roots of the four catch crop species and the mixture in five environments and across all environments. Shoot and root P contents were determined in mustard, phacelia, oat and clover cultivated either alone or in a mixture ("Mix") of all four species in 2015, 2016 and 2017 in Asendorf and 2016 and 2017 in Triesdorf. Bars (upward: shoot, downward: root) show the mean -SD; n = 3 or 15 plots. Red dots show BLUEs across all environments; n = 15. In individual environments, BLUEs are shown for comparison. In individual environments, estimated means are extracted from model 1 and when followed by a common letter not significantly different at the 5% significance level; across environments estimated means are extracted from model 4 and when followed by a common letter not significantly different at the 5% significance level; n.s.=not significant. n.a.=not analyzed. "Root P: Total P" shows the ratio of root-bound P to P bound in the total plant biomass; n = 3 or 15 plots. The adjusted repeatability for species ("Rep") is given in the upper right panel.

package. In order to decompose the individual relative variance contributions of *cv, b, y, s, cvs, cvy, ys, by* and/or *bs* to the total variance in the respective datasets, we calculated the unadjusted repeatability (*R*) for *cv, b, y, s, cvs, cvy, ys, by* and/or *bs* from an updated model 4, treating all effects as random, with the following equation (Nakagawa and Schielzeth, 2010):

$$R_{cv/b/y/s/cvs/cvy/ys/by/bs} = \sigma^2_{cv/b/y/s/cvs/cvy/ys/by/bs} \Big/ \Big(\sigma^2_{cv} + \sigma^2_b + \sigma^2_y + \sigma^2_s + \sigma^2_{cvs}$$
$$+ \sigma^2_{cvy} + \sigma^2_{ys} + \sigma^2_{by} + \sigma^2_{bs} + \sigma^2_e \Big)$$

(1)

$R_{cv/b/y/s/cvs/cvy/ys/by/bs}$ … unadjusted repeatability for catch crop variant / block / year / site / catch crop variant-site interaction / catch crop variant-year interaction / year-site interaction / block-year interaction or block-site interaction effect in updated model 4;

$\sigma^2_{cv/b/y/s/cvs/cvy/ys/by/bs}$ … variance estimated from cv_i / b_j / y_k / s_l / cvs_{il} / cvy_{ik} / ys_{kl} / by_{jk} or bs_{jl} treated as random factor in updated model 4;

σ^2_e… residual error from e_{ijkl} in updated model 4.

In order to estimate how accurate biomass formation and N and P accumulation among catch crop variants can be measured in changing environments, we adjusted the repeatability, weighted for the number of

replicates, for the catch crop variant by the following equation (Nakagawa and Schielzeth, 2010):

$$Rep = \sigma_{cv}^2 \bigg/ \left(\sigma_{cv}^2 + \frac{\sigma_e^2}{n_{cv}} \right) \quad (2)$$

Rep… adjusted repeatability for catch crop variant; σ_{cv}^2… variance estimated from cv_i treated as random factor in updated model 4; σ_e^2… residual error from e_{ijkl} in updated model 4; n_v… number of replicates per catch crop variant (n = 15).

To compute the variance contributions of catch crop species (R_{cs}), block (R_b), year (R_y) and site (R_s) and respective interactions to the total variation in the datasets of N and P concentrations and root:shoot ratios in N and P concentrations in pure stands, we first sub-set the datasets for the cultivation form "pure". Then, random effect models as shown for updated model 4 were fitted, replacing the effect of catch crop variant (cv) by catch crop species (cs) in otherwise identical models. The unadjusted repeatability was calculated according to Eq. 1. We followed the same procedure to compute R_{cs} for the cultivation form "mixture", replacing catch crop variant (cv) by catch crop species (cs) and block (b) by mixture-plot (m) in otherwise identical variants of updated model 4.

Relations between N_{min} or P_{ex} concentrations in the soil before catch crop sowing (trait 1) and respective N or P accumulation in the plant material (trait 2) were calculated according to Piepho (2018). In our experimental design, data pairs from the same environment (=combination of year and site) may be expected to be more similar than those from different environments. Thus, we fitted multivariate linear mixed models allowing for heterogeneous variance using the sommer package (Covarrubias-Pazaran, 2016, 2018). In detail, we used models with compound symmetry plus diagonal covariance structures and unconstrained estimation of the variance and covariance components as suggested for multivariate heterogeneous variance models (model 5 as specified by Covarrubias-Pazaran in https://rdrr.io/cran/sommer/f/inst/doc/v4.sommer.gxe.pdf and https://rdrr.io/cran/sommer/f/vignettes/v1.sommer.quick.start.Rmd, both accessed on 14.3.2022); mmer[cbind(trait 1, trait 2) ~ 1; random= ~ vs(catch crop variant, Gtc=uncm(2)) + vs(ds(environment), catch crop variant, Gtc=uncm(2)); rcov= ~ vs(ds(environment), units, Gtc=uncm(2))]. However, whenever "soil N_{min}" was imputed as trait 1 this model did not converge. Thus, trait 1 "N_{min}" and traits 2 "shoot N", "root N" or "N root: N total", respectively (see Table 4), were analysed in separate models [mmer(trait 1 ~ 1 OR trait 2 ~ 1; random= ~catch crop variant + vs(ds(environment), catch crop variant); rcov= ~ vs(ds(environment), units)]. Residuals of the respective models were checked for normality based on diagnostic plots (Kozak and Piepho, 2018). Data were log-transformed when needed. Then, we extracted the respective best linear unbiased predictors (BLUPs) of each catch crop variant for soil N_{min}/P_{ex} and N/P accumulation traits and correlated them by Pearson's product-moment correlation as listed in Table 4. Additionally, we correlated residuals of the respective traits. This allowed separate consideration of environment effects apart from residual effects (Piepho, 2018). However, since sampling and field management in Asendorf 2015 deviated strongly from the other years (i.e., plowing before catch crop sowing, use of fiber mat cover, determination of soil N_{min} pools more than one month before catch crop sowing), data from that environment were excluded from the correlation studies.

3. Results

3.1. Root versus shoot biomass formation in different catch crop species

To compare nutrient fixation in the root and shoot biomass among different catch crop species, we adjusted the seeding densities to establish comparable shoot biomasses of the different species at harvest. In the same environment, the aboveground biomasses of the three nonlegumes were highly comparable, so that the BLUEs for the shoot

Table 4

Relations between soil N_{min} or P_{ex} stocks before sowing (Trait 1) and corresponding elements captured in the shoots, in the roots or in the root-to-total plant biomass ratio (Trait 2) of catch crop variants. Trait 1 data are taken from Table 2 and trait 2 data from Figs. 2 and 3. For each catch crop variant, BLUPs were computed as described in Section 2.5 and correlated by Pearson's product-moment correlation. Values show correlation estimates (r) and corresponding p values; n = 4. Note: Residuals were correlated for trait 1 = "N_{min}" and trait 2 = "N shoot" with $r_{resid} = 0.67$ and $p_{resid} < 0.001$, and for trait 1 = "Pex" and trait 2 = "P root" with $r_{resid} = -0.78$ and $p_{resid} < 0.001$.

Catch crop variant	Trait 1	Trait 2	r	p value
Mustard	N_{min}	N shoot	0.95	< 0.05
Mustard	N_{min}	N root	-0.59	0.41
Mustard	N_{min}	N root:N total	-0.78	0.22
Mustard	P_{ex}	P shoot	0.45	0.56
Mustard	P_{ex}	P root	-0.18	0.81
Mustard	P_{ex}	P root:P total	-0.93	0.07
Phacelia	N_{min}	N shoot	0.99	< 0.05
Phacelia	N_{min}	N root	-0.43	0.57
Phacelia	N_{min}	N root:N total	-0.68	0.31
Phacelia	P_{ex}	P shoot	0.56	0.43
Phacelia	P_{ex}	P root	-0.17	0.83
Phacelia	P_{ex}	P root:P total	0.13	0.87
Oat	N_{min}	N shoot	0.99	< 0.001
Oat	N_{min}	N root	-0.54	0.46
Oat	N_{min}	N root:N total	-0.69	0.31
Oat	P_{ex}	P shoot	0.59	0.40
Oat	P_{ex}	P root	0.69	0.31
Oat	P_{ex}	P root:P total	-0.59	0.40
Clover	N_{min}	N shoot	0.83	0.17
Clover	N_{min}	N root	-0.36	0.64
Clover	N_{min}	N root:N total	-0.63	0.37
Clover	P_{ex}	P shoot	0.80	0.20
Clover	P_{ex}	P root	-0.84	0.15
Clover	P_{ex}	P root:P total	-0.85	0.15
Mixture	N_{min}	N shoot	0.98	< 0.05
Mixture	N_{min}	N root	-0.63	0.37
Mixture	N_{min}	N root:N total	-0.78	0.22
Mixture	P_{ex}	P shoot	-0.33	0.67
Mixture	P_{ex}	P root	-0.99	< 0.01
Mixture	P_{ex}	P root:P total	-0.74	0.26

biomass of the nonlegumes across all environments ranged between 2.2 (mustard) and 2.4 (oat) t ha^{-1} (Fig. 1). The BLUE of clover was only 69–73% relative to the other species. This was because of the slow juvenile development of clover and could not be corrected by increasing the seeding density.

In two of the five environments, namely in Asendorf and in Triesdorf in 2017, shoot biomasses were very close to the BLUEs, while at both sites in 2016 shoot biomasses strongly deviated from them. In Asendorf in 2016, the catch crops produced between 1.7 (clover) and 2.0 (oat) times more shoot biomass than the BLUEs (Fig. 1). In contrast, in Triesdorf 2016 shoot biomasses were only 0.07 (oat) to 0.3 (mustard) times the BLUE. This points to a large impact of the environment on shoot biomass formation of the catch crops. Indeed, the year-site interaction explained a large part of the variation in shoot biomass with R_{ys} = 0.72 (S1 Table). Among all sites and years, the shoot biomass of mustard varied up to 6-fold, from 0.7 to 4.1 t dry matter ha^{-1} (Fig. 1). The highest shoot biomass was produced in Asendorf in 2016, followed by Asendorf in 2017, Triesdorf in 2017, Asendorf in 2015 and Triesdorf in 2016. In principle, the other catch crop species kept the same order but with a larger range of variation, namely, 9-fold in phacelia and ~24-fold in both oat and clover. The total shoot biomass of the catch crop mixture was affected similarly by the growth conditions as in pure stands but varied only ~3-fold over the test conditions, which was much less than that of any of the pure stands.

The root biomass was more variable among the nonlegume catch crops in pure stands than the shoot biomass, ranging from 2.7 (mustard) over 3.5 (phacelia) to 3.6 (oat) t ha^{-1} across all environments (Fig. 1). Again, clover showed with 0.9 t ha^{-1} lower biomass production than the

nonlegumes (p < 0.05). Compared to shoot biomass, which was close to the BLUEs in only two test environments, root biomass was often close to the BLUE of a respective species, namely in three environments in mustard and oat and in all five environments in clover. Thus, the adjusted repeatability for the species across all environments was higher for root than for shoot biomass. The constant order in biomass formation among environments, which was observed for shoots of all catch crops, was only in part present in the roots. For all species, the lowest root biomass was observed in Triesdorf in 2017; however, due to the extraordinarily high root mass of phacelia and oat in Asendorf in 2015, the two species presented a different order compared with mustard, whose root biomass was highest at both sites in 2016. Early tillage by plowing and the initial cultivation of catch crops under a fiber mat to increase soil temperatures, which was undertaken only in Asendorf 2015, may have favored root growth of phacelia and oat more than that of mustard. The large root contribution of phacelia to the mixture in Asendorf in 2015 also resulted in the highest total root biomass of the mixture at that site-year combination.

The ratio of root-to-total biomass (root+shoot) followed a similar pattern in all catch crops across environments, albeit with large variation among species. Depending on the test conditions, we observed two scenarios: In Asendorf in 2016 and 2017 as well as in Triesdorf in 2017, the root biomass of the nonlegumes ranged from 20% to 50% of the corresponding total biomass, whereas in Asendorf in 2015 and Triesdorf in 2016, the catch crops had greater root biomass, which accounted for 66–95% of the of total biomass (Fig. 1). Except for Asendorf in 2015, clover behaved similar to the nonlegumes but with smaller root-to-total biomass ratios. This finding suggests that the contribution of roots to the total biomass is predominantly subject to the growth conditions, which is supported by repeatabilities of $R_{ys} = 0.75$, and only $R_{cv} = 0.04$ for that of catch crop variant (S1 Table). These results also indicate that any inaccuracy inherent to the DNA-based root biomass determination must have been smaller than the variation imposed by growth conditions.

In mixed culture, mustard and phacelia dominated shoot and root biomass formation. Whenever one of those species could cope better with the present growth conditions, exceptionally high biomasses also translated into the mixture, such as in the case of phacelia root biomass in Asendorf in 2015 (Fig. 1). Thus, the BLUE of the total root or shoot biomass of the mixture across all environments was either larger or similar to that of the best performing single species. This suggests a high robustness of the mixture in biomass production under different growth conditions. Similar to that observed for nonlegumes in pure stands, the contribution of roots to the total biomass in the mixture followed the same environment-dependent variations, and in three of the five test conditions it ranked highest among all catch crop variants. Thus, the root biomass of the mixture repeatedly accounted for a larger share of the overall plant biomass formation compared to that of the single species.

3.2. Variation in the root and shoot nutrient concentrations over years and sites

Similar to shoot and root biomass, nutrient concentrations in the plant material also varied among catch crop species and environmental conditions. In pure stands, the N concentrations in the shoots of mustard varied between 13 and 45 mg N g^{-1} shoot DW over the five tested conditions (Table 3). N concentrations in the other nonlegumes phacelia and oat were nearly identical to those of mustard within individual environments, and varied across environments in all species. The highest N concentrations were found in Asendorf in 2015 with ~40 mg N per g DW, followed by Triesdorf in 2016, Asendorf in 2016, Asendorf in 2017 and Triesdorf in 2017 with ~13 mg N per g DW, thus showing another ranking compared with shoot biomass (Fig. 1). The root N concentrations of the nonlegumes were similar within an environment as well, and followed in principle the same environmental variation as the shoot N concentrations but varied within a lower range

of 5–30 mg N g^{-1} root (Table 3). Most likely due to N_2 fixation (Saia et al., 2014), clover showed less variation (~1.3-fold) in either organ among the test environments than the nonlegumes. Thus, in environments where the nonlegumes enriched less N (Asendorf and Triesdorf in 2017), clover had > 2x more N in its shoot and between 3.2 and 6.8x more N in its root tissue. Consequently, clover had root:shoot ratios of N concentrations close to 1, while in the non-legumes root:shoot ratios ranged around 0.4–0.5.

In mixed cultivation, N concentrations in the shoots of mustard were comparable or slightly lower than in pure stands, whereas N was enriched significantly (p < 0.05) or in trend in the shoots of phacelia and oat in four of the five test environments (Table 3). The N concentrations in the roots could be determined separately from those of the pure and mixed stands only in Asendorf and in Triesdorf in 2017. Repeatedly, oat had higher N concentrations in the roots when grown in the mixture, while clover accumulated less N. The ratios of root N to shoot N concentrations were largely comparable between both cultivation forms in all catch crops. Variation in this trait was more affected by the properties of the individual plant species (pure stand: $R_{cs}=0.44$, mixture: $R_{cs}= 0.41$) than by the environment (pure stand: $R_{csys}= 0.09$, mixture: $R_{csys} = 0.09$; S1 Table).

Phosphorus concentrations in the shoots varied larger among catch crop species than those of N. In pure stands, phosphorus concentrations in mustard varied from 2.5 to 7 mg g^{-1} shoot DW (Table 3). Clover was in a highly similar range, while the P concentrations of phacelia and oat were 120–180% higher than that of mustard and varied by 2.4- and 3.1-fold across environments, respectively. In principle, the shoot P concentrations followed the order Asendorf 2015 > Triesdorf 2016 > Asendorf 2017 = Triesdorf 2017 > Asendorf 2016 in all catch crops; therefore, the environment had a highly similar impact on the shoot P levels. The extraordinarily high shoot P concentrations in Asendorf in 2015 might be explained by the better soil P availability (Table 2) due to plowing to 30 cm soil depth that was performed approximately five months before catch crop sowing. In 2016 and 2017, the soil was not tilled deeper than 15–20 cm for at least 1.5 years before catch crop sowing at either site. The P concentrations in roots were less affected by growth conditions and varied only between 1.2- (mustard) and 2.2-fold (phacelia, Fig. 3). Notably, the root P concentrations followed a different species-specific pattern, as observed for the shoots. Phacelia and clover roots showed the greatest enrichment in P (2.5–5.4 mg P g^{-1} root DW), while oat roots contained only 1.3–2.5 mg P g^{-1} root DW. Accordingly, the root:shoot ratios of P concentrations were lowest in oat. Compared with the root:shoot ratios for N concentration, a larger part of the variation in the data was explained by the growth conditions (pure stand: $R_{cs}= 0.48$, $R_y= 0.15$, $R_s= 0.07$; mixture: $R_{cs}= 0.53$, $R_y= 0.15$, $R_s= 0.05$; S1 Table) and varied between 1.6- (in phacelia) and 2.3-fold (in oat) among environments.

Cultivation within the catch crop mixture especially increased the shoot P concentration of oat by up to 35% compared to the respective pure stands (Table 3). Also, the shoot P in phacelia tended to increase under cultivation in mixed vs. pure stands, while the shoot P in mustard and clover remained largely unaffected by the cultivation form under either test condition. As observed for N, the root-to-shoot P concentrations of individual species were also similar in pure stands and mixed cultures.

In summary, the concentrations of N and P in the shoots of catch crops were in particular affected by the year of cultivation and the year-site interaction (N - pure stand: $R_y= 0.56$, $R_{ys}= 0.17$; N - mixture: $R_y= 0.61$, $R_{ys}= 0.13$; P - pure stand: $R_y= 0.23$, $R_{ys}= 0.32$; P - mixture: $R_y= 0.20$, $R_{ys}= 0.19$; S1 Table). By contrast, variation in root concentrations of both elements were explained to a larger extent by the catch crop species (N - pure stand: $R_{cs}= 0.39$; N - mixture: $R_{cs}= 0.31$; P - pure stand: $R_{cs}= 0.44$; P - mixture: $R_{cs}= 0.47$). In mixed culture, oat and phacelia contained higher nutrient concentrations in the shoots and partially in the roots, while mustard and clover did not show benefits in terms of nutrient concentrations from co-cultivation with other species

(Table 3).

3.3. Nitrogen accumulation in the root and shoot biomass of catch crops in pure and mixed culture

To evaluate the agronomic potential for nutrient carry-over to the next crop, we compared the amount of biomass-bound N in the roots and shoots of each species in pure stand with the sum of biomass-bound N in the mixture over the five test conditions. We observed that the BLUEs for N accumulation in shoots were highly similar among species in pure stands, ranging from 47.1 kg N ha^{-1} in clover to 56.8 kg N ha^{-1} in phacelia (Fig. 2). The mixture was estimated to capture insignificantly more N, namely 66.4 kg N ha^{-1}. In individual environments, N accumulation deviated from the BLUEs: At the more fertile site in Asendorf, N capture by catch crop shoots was mostly above the BLUE, while in Triesdorf plants accumulated only 10–70% of the estimated N capture (disregarding clover in 2017). In Asendorf, the shoot biomass-N of the nonlegume species in pure stands stored between 34 and 113 kg ha^{-1} within the three years, while in Triesdorf the corresponding value was 35 kg N ha^{-1}. Interestingly, N accumulation in the shoot biomass of all nonlegume catch crop variants and the mixture increased when the soil N$_{min}$ stocks were higher before catch crops were sown. This expressed in significant positive correlations ($p < 0.05$) of the BLUPs for random environment effects (r; Table 4). A lack of a significant positive r was only observed for clover, which likely reflected the influence of N$_2$ fixation (Saia et al., 2014).

In roots, we calculated BLUEs between 21.8 and 84.5 kg ha^{-1} for N accumulation to a soil depth of 70 cm in pure stands and a BLUE of 105.8 kg N ha^{-1} in the mixture. The mixture captured thus significantly more N in roots than clover across all environments ($p < 0.05$; Fig. 2). Total N accumulated in the root biomass was less than that in the shoots in Asendorf in 2016, 2017 and Triesdorf in 2017 and amounted to 30 kg N ha^{-1}. In Asendorf in 2015 and Triesdorf in 2016, however, biomass-bound N in the roots was exceptionally high, thus contributing between 60 (Triesdorf in 2016) and > 200 kg N ha^{-1} (phacelia in Asendorf in 2015) to the overall biomass N. In Asendorf in 2015, we assume that the fiber mat cover may have led to higher soil temperatures and thus higher N mineralization in the topsoil, resulting in increased N uptake by those species with large topsoil root biomass (phacelia and oat; Heuermann et al., 2019). In that environment, high root biomass was coupled with high root N concentrations, while in Triesdorf in 2016, high root N concentrations primarily drove high root N stocks (Table 3). Obviously, root N dominated over shoot N in terms of contribution to the overall N content in the catch crop biomass in Triesdorf in 2016 (Fig. 2). However, over all test environments, there was no relation between the soil N$_{min}$ stocks and N accumulation by the catch crop roots (Table 4).

In general, the root biomass-bound N varied little among species when total root N was low. The root N of either species contributed approximately 17–33% to the total biomass-bound N (Fig. 2). When the root biomass-N was large, differences among species became evident, such as in Asendorf in 2015, when 71% of plant-bound N was found in the roots of phacelia, or in Triesdorf in 2016, when oat roots captured 89% of the overall biomass-N. In general, none of the species outperformed the others in terms of root N, and even clover dominated root-bound N once in Triesdorf in 2017. Thus, a high performance in accumulating N in the root biomass depended less on the species (R_{cv}=0.06) than on the growth conditions, in particular the year (R_y= 0.33) with a R_{cvy} of 0.40 (S1 Table). Correlations between the soil N$_{min}$ stocks and ratios of root N to total biomass-bound N showed a negative trend in all catch crops as well as in the mixture (Table 4). This indicates that all species tended to increase their N capture in roots relative to total N capture when soil N was limited.

Regarding the catch crop mixture, the root biomass N was either higher than in single species or among the highest but never > 20% less than the root N fixed by the best single catch crop. The mixture was estimated to accumulate the largest amounts of root N over all

environments (Fig. 2). A closer look revealed that this superior behavior of the mixture resulted from the N share contributed by different species, including phacelia and mustard as well as clover. When these three species accumulated the largest amounts of root biomass-N in pure culture, i.e., in Asendorf in 2015 and Triesdorf in 2016 and 2017, they also contributed the largest amounts to root biomass N in the mixture. Only oat failed to translate its large N pool into the root biomass (Asendorf in 2015 and Triesdorf in 2016) from the pure culture to the mixture.

3.4. Phosphorus accumulation in the root and shoot biomass of different catch crops

The accumulation of P by the different catch crops showed more variation than that of N and robustly followed a species-specific order. In four of the five environments, phacelia was most effective in aboveground P enrichment, followed by oat, mustard and clover (Fig. 3). Across all environments, phacelia and oat were estimated to accumulate 12.5 and 10.5 kg P ha^{-1}, respectively, while mustard and clover captured only 45–72% of that P. Belowground, the BLUE for P accumulation in clover was 4 kg P ha^{-1}, which was the least amount of P, while phacelia, mustard and oat enriched 10.5, 8.0 and 6.8 kg P ha^{-1}, respectively. In these species, the contribution of the roots to the overall biomass-bound P was between 23% and 52%, except for Triesdorf in 2016, when the roots fixed 3.5–5.7 times more P than the shoots. Since shoot biomasses were similar, at least among the nonlegumes, the P concentrations largely determined the species' ability for P enrichment. In all species, the shoot P accumulation was in trend positively associated with the soil P$_{ex}$ stocks before sowing (Table 4). Compared to N, however, the values for r were weaker. This may be related to the fact that, compared with the N$_{min}$ fraction, the P$_{ex}$ method Mehlich 3 not only directly yields plant-available inorganic P but can also extract organic and tightly soil-bound P forms that may not contribute to plant nutrition (Cade-Menun et al., 2018).

Phacelia was generally most efficient in belowground P accumulation (Fig. 3). In the dry year 2016, mustard followed phacelia in root P accumulation, while oat performed better in the humid autumn in Asendorf in 2017. In Triesdorf in 2017, there were no differences in belowground P contents among all pure stands. Similar to N, the root P accumulation and the ratio of root P to total plant P were not associated with soil P$_{ex}$ pools in any of the catch crop variants (Table 4).

In three out of four tested environments, the mixture captured most P in its root biomass (Fig. 3) and its estimated mean for root P across all environments was significantly higher than that of clover ($p < 0.05$). The total P contents in the mixed stand mainly depended on mustard and phacelia. In Asendorf, the total P accumulation by the mixture was always as high as that in the best-performing pure stand, while in Triesdorf, the mixture even outperformed the pure stands. Thus, the mixture showed the most robust potential for P carry-over in both, roots and shoots.

4. Discussion

In recent years, German legislation has increased the number of legal restrictions for nutrient and fertilizer input in agricultural plant production. Amongst others, these restrictions are based on the site-specific monitoring of N and P fertilizer inputs and provide a guide value for P inputs and a threshold for N, an upper limit for N application via organic and organic-mineral fertilizers of 170 kg ha^{-1} or the reduction of the N fertilizer requirement by 20% in areas with high nitrate levels in the groundwater (German Federal Ministry of Food and Agriculture, 2020). Against this background, catch crops have become an instrumental component in planning nutrient budgets in crop rotations. Therefore, understanding the nutrient carry-over potential of individual catch crops is essential for performing reliable estimates of fertilizer regimes in target crops. Here, we place emphasis on the contribution of roots and

validate the hypothesis that catch crop mixtures show superior robustness in N and P capture across varying environments than pure stands, especially when root biomass is considered.

4.1. Impact of environmental conditions on the nutrient pools captured by roots

The present field study found that the roots accumulated ~20–105 kg N and 4–15 kg P ha^{-1} (BLUEs) in their biomass, which accounts for 26–46% of the total plant N and 36–48% of the total plant P (Fig. 2, Fig. 3). These numbers emphasize the importance of the belowground nutrient pool for the overall nutrient carry-over potential of catch crops. Considering that nutrient accumulation is a product of biomass formation and nutrient concentration, the ratio of root biomass to total plant biomass has a strong impact on the below-ground nutrient pool. Across the five environments tested here, this ratio was less dependent on the catch crop species than on the environment (Fig. 1, S1 Table). Large variations in biomass formation and root:shoot ratios across environments are common in many species and have been revealed by different approaches that estimate root and shoot biomass separately, such as allometric methods in woody plants (Xing et al., 2019), ^{13}C isotope labeling (Subedi et al., 2006) or metadata analyses in annual plants (Hu et al., 2018). Relative to the biomass, nutrient concentrations in the shoot and root tissues varied less with environmental conditions and thus represent the more stable factor when estimating the nutrient transfer potential of catch crops (Fig. 2, Fig. 3).

Here, we observed considerable variation in the contribution of the root N pool to total biomass-N, which ranged between 17% and 71% in oat or phacelia at the experimental station in Asendorf or between 17% and 33% or 76% and 89% over the two years in Triesdorf (Fig. 4). These differences were apparently not driven by the soil N$_{min}$ status alone (Table 4). In Triesdorf in 2016, high root N:total plant N ratios resulted from high root biomasses, which were consistent with the extremely low shoot biomasses of all species (Fig. 1, Fig. 2). In this vegetation period, low precipitation together with comparatively low soil N$_{min}$ levels on the sandy clay/loam soil in Triesdorf (Table 1, Table 2) might have forced plants to invest more resources into root foraging for water (Xu et al., 2015) and nutrients at the cost of shoot development (Giehl and von Wirén, 2014). Also Komainda et al. (2016) reported that the contribution of the root-N pool to the total biomass-N captured in rye (*Secale cereale*) and Italian ryegrass (*Lolium multiflorum)* increased from ~15–20% to ~35–40% when the root:shoot biomass ratio increased under lower rainfall (Komainda et al., 2016). Notably, low precipitation also occurred in Asendorf in 2016; however the soil N$_{min}$ stocks were very large. There, the share of roots in total plant N accumulation was only ~20% (Fig. 2, Table 1, Table 2). This observation suggests that in nonlegumes the contribution of root-bound N to overall biomass-N increases if both, soil N$_{min}$ and precipitation, are low. Then, the root N pools with their ~75 to ~90% share of total plant N (Triesdorf in 2016) gained even more importance for the overall N transfer potential of a catch crop.

The extraordinarily high root:shoot biomass ratios in Triesdorf in 2016 also drove the large contribution of root-bound P to overall plant P (Table 2, Table 3, Table 1, Fig. 1, Fig. 3). Roots accounted for 77–89% of the total plant P, while in 2017, only 33–47% of the overall plant P was bound in roots (Fig. 3). During the dry season of 2016 in Asendorf, the root biomass of mustard and its P content increased relative to the shoot, while less P was captured in the roots of phacelia and oat than in the shoots. One reason might be that in the dry year, diffusion of soil phosphate to the root surface was low (Lambers et al., 2006), resulting in lower P acquisition by roots in species whose P enrichment is usually high (Fig. 3, Eichler-Löbermann et al., 2008). Notably, under these dry conditions in Asendorf, mustard had the highest root P concentration among the nonlegumes, while in the other test environments, phacelia showed superior root P concentrations (Table 3). The high performance of phacelia and oat in root P accumulation may also be due to a

relatively high root length density, which enhances the soil volume that can be exploited, especially for nutrients that reach the root surface via diffusion, such as P and potassium (Wendling et al., 2016). Across environments, the total root P accumulation followed the pattern of P concentrations (Table 3) rather than root biomass (Fig. 1). Therefore, we conclude that high P concentrations in the root tissue are a dominant factor or even a prerequisite for high P capture by the roots and that a certain availability of water is required for P acquisition by high P-enriching species.

4.2. Synergistic effects allow catch crop mixtures to outperform pure cultures

Since the amount of nutrients bound in the total biomass of purely cultivated catch crops was strongly affected by the growth conditions, it is difficult to recommend a single species for maximum nutrient capture (Fig. 2, Fig. 3). Therefore, we determined the nutrient uptake of the four-species mixture, which is technically more complicated than that of pure stands because discriminating the of roots of individual species is challenging. Nonetheless, by using different methods to assign roots to individual component species, previous studies have shown that some species (Polley et al., 1992; Mommer et al., 2010) and even genotypes (Streit et al., 2019) are more competitive in mixtures than others, that competitor strength can vary during the duration of plant cultivation (Robinson et al., 2010) and that growth conditions have an impact on the relative increase or decrease in the root and shoot biomass of individual component species (Mommer et al., 2010; Streit et al., 2019). Also here, the environment affected strongly the individual contributions of the root or shoot biomass by mustard, phacelia, oat or clover to the total biomass of the catch crop mixture (Fig. 1). Nonetheless, we identified mustard and phacelia as more competitive at our experimental sites than the other two species. In fact, the biomass formation of clover and oat in the mixture was largely repressed by mustard and phacelia (Heuermann et al., 2019).

Mustard and phacelia together contributed ~40–95% of the total N fixed in the roots and ~60 to almost 100% of the total N captured in shoots of the mixture (Fig. 2). Since mustard showed similar or even lower N concentrations in the mixture compared to pure stands (Table 3), its high performance was mainly related to high biomass formation (Fig. 1). In phacelia, the N concentrations were significantly higher (p < 0.05) or showed a stronger trend in the mixture (Table 3), indicating that cultivation of phacelia in a mixture with the other investigated species increases its N acquisition efficiency. A similar observation was made regarding P accumulation (Table 3, Fig. 3). In mixtures, synergistic effects arising from the co-cultivation of different species may improve nutrient acquisition, e.g., by microbiome changes that mobilize nutrients, by establishing symbiotic mycorrhizae that help to acquire P and may even transfer N from one to the other species, by enhancing soil P mobilization when organic acids or P-mobilizing compounds are released or when component species acidify the rhizosphere (Hinsinger et al., 2011). The extent to which these mechanisms played a role in our mixture remains to be investigated. Nonetheless, we expect the contribution of oat and clover to P mobilization via rhizosphere acidification and the release of organic acids or P-mobilizing compounds as rather low because their root biomass formation was strongly repressed in the mixture (Fig. 1). Furthermore, we consider functional niche differentiation among species (Mommer et al., 2010) of less priority here, as the two dominating species mustard and phacelia shared the same root niches along vertical soil profiles (Heuermann et al., 2019).

Across all tested conditions, we found that nutrient capture by the mixture was similar or even better than that in the best-performing pure stand (Fig. 2, Fig. 3). In general, those species that coped better with varying environmental conditions in their pure stands also contributed most to the mixture. When mustard and phacelia accumulated equal amounts of nutrients, they also contributed proportionally to nutrient

fixation in the mixture. However, when environmental conditions were better for nutrient accumulation in oat or clover than in the other two species as in 2017, the former species also made large contributions to nutrient capture of the mixture (Fig. 2, Fig. 3). This observation supports the view that mixtures of different species exhibit higher durability and resilience to unfavorable growth conditions than pure stands due to compensatory effects among species (Wortman et al., 2012; Elhakeem et al., 2019; Khan and McVay, 2019). Moreover, our results indicate superior robustness of catch crop mixtures regarding nutrient carry-over potential via both the shoot-bound and root-bound nutrient pools. Thus, the root-bound nutrient pools of catch crop mixtures attain an important function in sustainable agricultural management practices to optimize and fully exploit nutrient carry-over potentials in variable environments, which become more frequent with global climate change.

4.3. Importance of nutrient capture by the mixture for nutrient management in crop rotations

Residual plant N, which remains in the field after harvest, is a major problem in the cultivation of crops, as losses to the hydrosphere, especially in the form of leachable nitrate, can cause environmental problems such as eutrophication or hypoxia (Galloway and Cowling, 2002). Postharvest N_{min} pools in the soil have been reported for various crop species and range, for example, from 24 to 45 (McEwen et al., 1990) over ~65 (Maidl et al., 1991) up to 103 kg N ha^{-1} in wheat (Sieling and Christen, 2015) or from 43 to 67 (McEwen et al., 1990) to 148 or 121 kg N ha^{-1} in field bean (*Vicia faba*) and pea (*Pisum sativum*; Maidl et al., 1991), respectively. For potato (*Solanum tuberosum*), soil nitrate pools of 35–159 kg ha^{-1} were measured (Widdowson et al., 1987), while maize (*Zea mays*) left 41–164 (Ferguson et al., 2002) or even 97–208 kg N ha^{-1} of soil nitrate after harvest (Liu et al., 2017). In the present study, we found that the mixture of mustard, phacelia, oat and clover was more stable in terms of nutrient accumulation than the individual catch crop species (Fig. 2, Fig. 3). Across all environments, the mixture was estimated to accumulate 172 kg N ha^{-1} in its total plant biomass. Considering that the residual N after the abovementioned crops was mostly below the BLUE determined here for the 4-species mixture, the present mixture represents an efficient tool to manage residual N from a wide range of crop species.

Total P accumulation in the biomass of the catch crop mixture was estimated to be 29 kg h^{-1} (Fig. 3). The advantage of P captured in catch crop biomass is its plant-available form, which generates a readily accessible P source for the following crop, especially when compared to inorganic rock phosphate (Eichler-Löbermann et al., 2008). Several studies reported the uptake of ~10–35 kg P ha^{-1} by various crop species during their cultivation, e.g. 26.8–34.4 (Krey et al., 2013) or 35.2 kg P ha^{-1} in maize (Setiyono et al., 2010), 9.4–12.1 (Rehim et al., 2015) or 13.6–33.4 kg P ha^{-1} in wheat (Sandaña and Pinochet, 2014), 4.4–15.8 (Poulton et al., 2013) or 23.5–28.6 kg P ha^{-1} in barley (Nelissen et al., 2015), 9.9–28.9 (Sandaña, 2016) or 16.9–23.3 kg P ha^{-1} in potato (Zarzecka and Gugała, 2010) and 4–14 (Ryan and Angus, 2003) or 15.5–29.8 kg P ha^{-1} in field pea (Sandaña and Pinochet, 2014). Actually, these numbers are consistent with the total P captured by the catch crop mixture in our study (Fig. 3). Thus, the present mixture of mustard, phacelia, oat and clover can almost fully meet the total P demand of a major following target crops. However, it must be kept in mind that the degradability of the plant material, as determined e.g. by its C:N ratio, also determines its mineralization kinetics and thus the amount of nutrients released over time (Thorup-Kristensen et al., 2003). In this regard, catch crop mixtures promise shorter degradation periods, especially if they contain legumes with narrow C:N ratios and species of different metabolic compositions that may balance and increase microbial activity.

Research Data

The datasets generated and/or analyzed during the current study are available in the e!DAL repository (Arend et al., 2014) under https://doi.org/10.5447/ipk/2021/6.

CRediT authorship contribution statement

Diana Heuermann: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft; **Norman Gentsch**: Investigation, Writing – review & editing; **Georg Guggenberger**: Writing – review & editing; **Barbara Reinhold-Hurek**: Funding acquisition, Writing – review & editing; **Dörte Schweneker**: Investigation, **Ulf Feuerstein**: Resources, Writing – review & editing; **Marc Christian Heuermann**: Formal analysis; **Jonas Groß**: Investigation; **Robin Kümmerer**: Investigation, Writing – review & editing; **Bernhard Bauer**: Writing – review & editing; **Nicolaus von Wirén**: Project administration, Conceptualization, Methodology, Writing – original draft.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126504.

References

Arend, D., Lange, M., Chen, J., Colmsee, C., Flemming, S., Hecht, D., Scholz, U., 2014. e! DAL – a framework to store, share and publish research data. BMC Bioinform. 15, 214. https://doi.org/10.1186/1471-2105-15-214.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Cade-Menun, B.J., Elkin, K.R., Liu, C.W., Bryant, R.B., Kleinman, P.J.A., Moore Jr., P.A., 2018. Characterizing the phosphorus forms extracted from soil by the Mehlich III soil test. Geochem. Trans. 19 https://doi.org/10.1186/s12932-018-0052-9.

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus: global food security and food for thought. Glob. Environ. Change 19, 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009.

Covarrubias-Pazaran, G., 2016. Genome-assisted prediction of quantitative traits using the R package sommer. PLOS One 11, e0156744. https://doi.org/10.1371/journal.pone.0156744.

Covarrubias-Pazaran, G., 2018. Software update: moving the R package *sommer* to multivariate mixed models for genome-assisted prediction. bioRxiv, 354639. https://doi.org/10.1101/354639.

Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245, 35–47. https://doi.org/10.1023/a:1020809400075.

Eggert, K., von Wirén, N., 2013. Dynamics and partitioning of the ionome in seeds and germinating seedlings of winter oilseed rape. Metallomics 5, 1316–1325. https://doi.org/10.1039/c3mt00109a.

Eichler-Löbermann, B., Köhne, S., Kowalski, B., Schnug, E., 2008. Effect of catch cropping on phosphorus bioavailability in comparison to organic and inorganic

fertilization. J. Plant Nutr. 31, 659–676. https://doi.org/10.1080/01904160801926517.

Elhakeem, A., van der Werf, W., Ajal, J., Lucà, D., Claus, S., Vico, R.A., Bastiaans, L., 2019. Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. Agric. Ecosyst. Environ. 285, 106627 https://doi.org/10.1016/j.agee.2019.106627.

Ferguson, R., Hergert, G.W., Schepers, Js, Gotway, C.A., Cahoon, J., Peterson, T.A., 2002. Site-specific nitrogen management of irrigated maize: yield and soil residual nitrate effects. Soil Sci. Soc. Am. J. 66. https://doi.org/10.2136/sssaj2002.0544.

Galloway, J.N., Cowling, E.B., 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31, 64–71. https://doi.org/10.1579/0044-7447-31.2.64.

Gentsch, N., Boy, J., Batalla, J.D.K., Heuermann, D., von Wirén, N., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Céspedes, F.C., Guggenberger, G., 2020. Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. Biol. Fertil. Soils 56, 943–957. https://doi.org/10.1007/s00374-020-01475-8.

German Federal Ministry of Food and Agriculture, 2020. Verordnung zur Änderung der Düngeverordnung und anderer Vorschriften (DüVÄndV 2020).

Giehl, R.F.H., von Wirén, N., 2014. Root nutrient foraging. Plant Physiol. 166, 509–517. https://doi.org/10.1104/pp.114.245225.

Haling, R.E., Simpson, R.J., McKay, A.C., Hartley, D., Lambers, H., Ophel-Keller, K., Wiebkin, S., Herdina, Riley, I.T., Richardson, A.E., 2011. Direct measurement of roots in soil for single and mixed species using a quantitative DNA-based method. Plant Soil 348, 123–137. https://doi.org/10.1007/s11104-011-0846-3.

Heuermann, D., Gentsch, N., Boy, J., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Guggenberger, G., von Wirén, N., 2019. Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils. Sci. Rep. 9, 11531. https://doi.org/10.1038/s41598-019-48060-0.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., Tang, X., Zhang, F., 2011. P for two, sharing a scarce resource: soil phosphorus acquisition in the rhizosphere of intercropping species. Plant Physiol. 156, 1078–1086. https://doi.org/10.1104/pp.111.175331.

Hirte, J., Leifeld, J., Abiven, S., Oberholzer, H.-R., Hammelehle, A., Mayer, J., 2017. Overestimation of crop root biomass in field experiments due to extraneous organic matter. Front. Plant Sci. 8 https://doi.org/10.3389/fpls.2017.00284.

Hu, T., Sørensen, P., Wahlström, E., Chirinda, N., Sharif, B., Li, X., Olesen, J., 2018. Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass. Agric. Ecosyst. Environ. 251, 141–148. https://doi.org/10.1016/j.agee.2017.09.024.

Huang, C.Y., Kuchel, H., Edwards, J., Hall, S., Parent, B., Eckermann, P., Herdina, Hartley, D.M., Langridge, P., McKay, A.C., 2013. A DNA-based method for studying root responses to drought in field-grown wheat genotypes. Sci. Rep. 3, 3194. https://doi.org/10.1038/srep03194.

Hupe, A., Schulz, H., Bruns, C., Joergensen, R.G., Wichern, F., 2016. Digging in the dirt – inadequacy of belowground plant biomass quantification. Soil Biol. Biochem. 96, 137–144. https://doi.org/10.1016/j.soilbio.2016.01.014.

Jensen, T., 2006. Nitrogen fertilizer, forms and methods of application. In: Proceedings of the Irrigated Crop Production Update Conference. Lethbridge, Alberta, Canada, pp. 21–26.

Khan, Q.A., McVay, K.A., 2019. Productivity and stability of multi-species cover crop mixtures in the northern great plains. Agron. J. 111, 1817–1827. https://doi.org/10.2134/agronj2018.03.0173.

Koefender, J., Schoffel, A., Manfio, C., Golle, D., 2016. Biomass and nutrient cycling by winter cover crops. Rev. Ceres 63, 816–821. https://doi.org/10.1590/0034-737x201663060010.

Komainda, M., Taube, F., Kluß, C., Herrmann, A., 2016. Above- and belowground nitrogen uptake of winter catch crops sown after silage maize as affected by sowing date. Eur. J. Agron. 79, 31–42. https://doi.org/10.1016/j.eja.2016.05.007.

Kozak, M., Piepho, H.-P., 2018. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J. Agron. Crop Sci. 204, 86–98. https://doi.org/10.1111/jac.12220.

Kramberger, B., Gselman, A., Podvršnik, M., Kristl, J., Lešnik, M., 2013. Environmental advantages of binary mixtures of *Trifolium incarnatum* and *Lolium multiflorum* over individual pure stands. Plant Soil Environ. 59, 22–28. https://doi.org/10.17221/223/2012-PSE.

Krey, T., Vassilev, N., Baum, C., Eichler-Lobermann, B., 2013. Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. Eur. J. Soil Biol. 55, 124–130. https://doi.org/10.1016/j.ejsobi.2012.12.007.

Kuo, S., Sainju, U.M., Jellum, E.J., 1997. Winter cover cropping influence on nitrogen in soil. Soil Sci. Soc. Am. J. 61, 1392–1399. https://doi.org/10.2136/sssaj1997.03615995006100050016x.

Kutschera, L., Lichtenegger, E., Sobotik, M., 2009. Wurzelatlas Der Kulturpflanzen Gemäßigter Gebiete Mit Arten Des Feldgemüsebaues. DLG Verlag, Frankf. /Main.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J., 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann. Bot. 98, 693–713. https://doi.org/10.1093/aob/mcl114.

Lenth, R.V., 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.0. ⟨https://CRAN.R-project.org/package=emmeans⟩.

Liu, Z., Chen, Z., Ma, P., Meng, Y., Zhou, J., 2017. Effects of tillage, mulching and N management on yield, water productivity, N uptake and residual soil nitrate in a long-term wheat-summer maize cropping system. Field Crops Res. 213, 154–164. https://doi.org/10.1016/j.fcr.2017.08.006.

Lynch, J.P., 2019. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. N. Phytol. 223, 548–564. https://doi.org/10.1111/nph.15738.

Maidl, F.X., Suckert, J., Und, R.F., Fischbeck, G., 1991. Standorterhebungen zur Stickstoffdynamik nach Anbau von Körnerleguminosen. J. Agron. Crop Sci. 167, 259–268. https://doi.org/10.1111/j.1439-037X.1991.tb00873.x.

McEwen, J., Darby, R.J., Hewitt, M.V., Yeoman, D.P., 1990. Effects of field beans, fallow, lupins, oats, oilseed rape, peas, ryegrass, sunflowers and wheat on nitrogen residues in the soil and on the growth of a subsequent wheat crop. J. Agric. Sci. 115, 209–219. https://doi.org/10.1017/S0021859600075146.

Mommer, L., Van Ruijven, J., De Caluwe, H., Smit-Tiekstra, A.E., Wagemaker, C.A.M., Joop Ouborg, N., Bögemann, G.M., Van Der Weerden, G.M., Berendse, F., De Kroon, H., 2010. Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. J. Ecol. 98, 1117–1127. https://doi.org/10.1111/j.1365-2745.2010.01702.x.

Nakagawa, S., Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol. Rev. 85, 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x.

Nelissen, V., Ruysschaert, G., Manka'Abusi, D., D'Hose, T., De Beuf, K., Al-Barri, B., Cornelis, W., Boeckx, P., 2015. Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. Eur. J. Agron. 62, 65–78. https://doi.org/10.1016/j.eja.2014.09.006.

Paynel, F., Cliquet, J.B., 2003. N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds. Agronomie 23, 503–510. https://doi.org/10.1051/agro:2003022.

Piepho, H.-P., 2018. Allowing for the structure of a designed experiment when estimating and testing trait correlations. J. Agric. Sci. 156, 59–70. https://doi.org/10.1017/S0021859618000059.

Polley, H.W., Johnson, H.B., Mayeux, H.S., 1992. Determination of root biomasses of three species grown in a mixture using stable isotopes of carbon and nitrogen. Plant Soil 142, 97–106. https://doi.org/10.1007/BF00010179.

Poulton, P.R., Johnston, A.E., White, R.P., 2013. Plant-available soil phosphorus. Part I: the response of winter wheat and spring barley to Olsen P on a silty clay loam. Soil Use Manag. 29, 4–11. https://doi.org/10.1111/j.1475-2743.2012.00450.x.

R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Redin, M., Recous, S., Aita, C., Chaves, B., Pfeifer, I.C., Bastos, L.M., Pilecco, G.E., Giacomini, S.J., 2018. Root and shoot contribution to carbon and nitrogen inputs in the topsoil layer in no-tillage crop systems under subtropical conditions. Rev. Bras. Cienc. Solo 42. https://doi.org/10.1590/18069657rbcs20170355.

Rehim, A., Hussain, M., Hussain, S., Noreen, S., Doğan, Muhammad, Z.-U.-H., 2015. Band application of phosphorus with farm manure improves phosphorus use efficiency, productivity and net returns of wheat on sandy clay loam soil. Turk. J. Agric. 40 https://doi.org/10.3906/tar-1505-133.

Reich, P.B., Tilman, D., Naeem, S., Ellsworth, D.S., Knops, J., Craine, J., Wedin, D., Trost, J., 2004. Species and functional group diversity independently influence biomass accumulation and its response to CO_2 and N. Proc. Natl. Acad. Sci. USA 101, 10101–10106. https://doi.org/10.1073/pnas.0306602101.

Robinson, D., Davidson, H., Trinder, C., Brooker, R., 2010. Root–shoot growth responses during interspecific competition quantified using allometric modelling. Ann. Bot. 106, 921–926. https://doi.org/10.1093/aob/mcq186.

Ryan, M.H., Angus, J.F., 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. Plant Soil 250, 225–239. https://doi.org/10.1023/A:1022839930134.

Saia, S., Amato, G., Frenda, A.S., Giambalvo, D., Ruisi, P., 2014. Influence of arbuscular mycorrhizae on biomass production and nitrogen fixation of berseem clover plants subjected to water stress. PLOS One 9, e90738. https://doi.org/10.1371/journal.pone.0090738.

Sandaña, P., 2016. Phosphorus uptake and utilization efficiency in response to potato genotype and phosphorus availability. Eur. J. Agron. 76, 95–106. https://doi.org/10.1016/j.eja.2016.02.003.

Sandaña, P., Pinochet, D., 2014. Grain yield and phosphorus use efficiency of wheat and pea in a high yielding environment. J. Soil Sci. 14. https://doi.org/10.4067/S0718-95162014005000076.

Setiyono, T.D., Walters, D.T., Cassman, K.G., Witt, C., Dobermann, A., 2010. Estimating maize nutrient uptake requirements. Field Crops Res. 118, 158–168. https://doi.org/10.1016/j.fcr.2010.05.006.

Sieling, K., 2019. Improved N transfer by growing catch crops – a challenge. J. Kult. 71 https://doi.org/10.5073/jfk.2019.06.01.

Sieling, K., Christen, O., 2015. Crop rotation effects on yield of oilseed rape, wheat and barley and residual effects on the subsequent wheat. Arch. Acker Pflanzenbau Bodenkd. 61, 1531–1549. https://doi.org/10.1080/03650340.2015.1017569.

Streit, J., Meinen, C., Nelson, W.C.D., Siebrecht-Schöll, D.J., Rauber, R., 2019. Above- and belowground biomass in a mixed cropping system with eight novel winter faba bean genotypes and winter wheat using FTIR spectroscopy for root species discrimination. Plant Soil 436, 141–158. https://doi.org/10.1007/s11104-018-03904-y.

Subedi, K., Ma, B.L., Liang, B.C., 2006. New method to estimate root biomass in soil through root-derived carbon. Soil Biol. Biochem. 38, 2212–2218. https://doi.org/10.1016/j.soilbio.2006.01.027.

Sullivan, D.M., Andrews, N., 2012. Estimating plant-available nitrogen release from cover crops, Oregon State University fact sheet.

Thorup-Kristensen, K., 2001. Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant Soil 230, 185–195. https://doi.org/10.1023/A:1010306425468.

Thorup-Kristensen, K., 2006. Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. Plant Soil 288, 233–248. https://doi.org/10.1007/s11104-006-9110-7.

Thorup-Kristensen, K., Magid, J., Jensen, L.S., 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. Adv. Agron. 79, 227–302. https://doi.org/10.1016/S0065-2113(02)79005-6.

Wendling, M., Büchi, L., Amossé, C., Sinaj, S., Walter, A., Charles, R., 2016. Influence of root and leaf traits on the uptake of nutrients in cover crops. Plant Soil 409, 419–434. https://doi.org/10.1007/s11104-016-2974-2.

Widdowson, F.V., Penny, A., Darby, R.J., Bird, E., Hewitt, M.V., 1987. Amounts of NO3-N and NH4-N in soil, from autumn to spring, under winter wheat and their relationship to soil type, sowing date, previous crop and N uptake at Rothamsted, Woburn and Saxmundham, 1979–85. J. Agric. Sci. 108, 73–95. https://doi.org/10.1017/S0021859600064145.

Wortman, S.E., Francis, C.A., Lindquist, J.L., 2012. Cover crop mixtures for the western corn belt: opportunities for increased productivity and stability. Agron. J. 104, 699–705. https://doi.org/10.2134/agronj2011.0422.

WRB, I.W.G., 2015. World Reference Base for Soil Resources 2014, update 2015. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resource. Report No. 106. FAO, Rome.

Xavier, F., Oliveira, J., Silva, M., 2017. Decomposition and nutrient release dynamics of shoot phytomass of cover crops in the Recôncavo Baiano. Rev. Bras. Cienc. Solo 41. https://doi.org/10.1590/18069657rbcs20160103.

Xing, D., Bergeron, J.A.C., Solarik, K.A., Tomm, B., Macdonald, S.E., Spence, J.R., He, F., 2019. Challenges in estimating forest biomass: use of allometric equations for three boreal tree species. Can. J. For. Res. 49, 1613–1622. https://doi.org/10.1139/cjfr-2019-0258.

Xu, W., Cui, K., Xu, A., Nie, L., Huang, J., Peng, S., 2015. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. Acta Physiol. Plant. 37, 9. https://doi.org/10.1007/s11738-014-1760-0.

Yu, P., He, X., Baer, M., Beirinckx, S., Tian, T., Moya, Y.A.T., Zhang, X., Deichmann, M., Frey, F.P., Bresgen, V., Li, C., Razavi, B.S., Schaaf, G., von Wirén, N., Su, Z., Bucher, M., Tsuda, K., Goormachtig, S., Chen, X., Hochholdinger, F., 2021. Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. Nat. Plants 7, 481–499. https://doi.org/10.1038/s41477-021-00897-y.

Zaniewicz-Bajkowska, A., Rosa, R., Kosterna, E., Franczuk, J., 2013. Catch crops for green manure: biomass yield and macroelement content depending on the sowing date. Acta Sci. Pol. Hortorum Cultus 12, 65–79.

Zarzecka, K., Gugała, M., 2010. Content and uptake of phosphorus and calcium with the yield of potato tubers depending on cultivation operations. J. Elementol. 15 https://doi.org/10.5601/jelem.2010.15.2.385-392.

Ziadi, N., Tran, T.S., 2006. Mehlich 3-extractable elements. Frontiers In Ecology And The Environment Soil Sampling And Methods Of Analysis. CRC Press.