



Plant and soil N of different winter cover crops as green manure for subsequent organic white cabbage

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Abstract Leguminous cover crops used as green manures can reduce fertilizer inputs by supplying nitrogen (N) via mineralization of incorporated N-rich biomass derived from biological N₂ fixation. In a multi-year trial at three locations in Germany, the effects of leguminous, non-leguminous and mixed green manure crops on the yield of the subsequent cash crop white cabbage (*Brassica oleracea* convar. *capitata* var. *alba*) were investigated. The winter

cover crop treatments were forage rye (*Secale cereale* L.), a mixture of forage rye with winter Hungarian vetch (*Vicia pannonica* Crantz), sole-cropped winter Hungarian vetch, winter pea (*Pisum sativum* L.), and winter faba bean (*Vicia faba* L.) with bare soil as a control. Sole-cropped legumes showed higher marketable cabbage head yields (head weight > 1.0 kg) compared to the other cover crop treatments, with 25.5, 25.9 and 28.1 Mg ha⁻¹ for vetch, pea and faba bean, respectively. The aboveground biomass of the legume winter cover crop treatments had higher N offtakes with 185, 177 and 159 kg N ha⁻¹ for vetch, pea and faba bean, respectively, with significantly lower carbon (C)/N ratios compared to rye and rye with vetch. The constant C/N ratio of the aboveground biomass of leguminous cover crops throughout the growing period indicates that the optimum incorporation date to achieve high N mineralization rates is less time dependent in leguminous compared to non-leguminous cover crops. The results of the present study show that leguminous winter cover crops do not reduce the soil N availability for a succeeding high N demanding cabbage crop resulting in yields comparable to agricultural practice without winter cover crops.

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Introduction

In organic horticulture, the source and amount of permitted commercial fertilizers and farm manures are limited by the restrictions of organic regulations, e.g. the Regulation (EU) 2018/848 of the European Parliament and of the Council (European Parliament and European Council 2022) and additionally by private standards of organic associations (Bio Austria 2021; Bioland eV 2023; Demeter eV 2021). However, the demand for organically produced vegetables is constantly increasing at the national, EU-, and international level (European Commission 2019; Willer and Lernoud 2019; BLE 2020). Some vegetables, e.g. cabbage, zucchini or broccoli, have high nutrient requirements within a short growing period in comparison to arable crops (Feller and Fink 2005). Therefore, the supply of vegetables with a high nutrient demand, especially nitrogen (N), requires a well-functioning fertilisation management. Due to the specialization and intensification of intensive vegetable production, farms often have little to no animal husbandry to generate solid farmyard manure within the farming system. Consequently, large quantities of farmyard manures, as well as commercial fertilizers, are purchased from outside the farm. Farmyard manures are mainly multi-nutrient fertilizers with a nutrient stoichiometry that often does not match the nutrient offtake of the vegetable crops (Zikeli et al. 2017; Möller 2018). Therefore, fertilization with manure may lead to nutrient imbalances, particularly phosphorus (P) oversupply in the soil (Cooper et al. 2018; Möller 2018). Additionally, stockless organic horticultural farms commonly use N-dominant commercial fertilizers, which are often waste materials from conventional food production (Voogt et al. 2011; Möller and Schultheiß 2014). Keeping balanced N/P ratios in the overall fertilization scheme in mind, solid animal manures and composts can provide a maximum of 15–25% of the N demand of any cropping system (Möller 2018). Therefore, fertilisation of 100 kg N via composts means that approximately 400 kg N should be supplemented by sources nearly free of P, e.g. biological N_2 fixation (BNF). Measures that enhance N inputs via BNF and improve internal N cycling in organic horticulture need to be optimized to reduce the need for external fertilizers and to adjust nutrient imbalances as a consequence of the use of external inputs (Möller 2018).

The overall approach to maintain soil fertility in organic farming is based on a crop rotation containing cover crops that provide ecosystem services and the incorporation of crop residues as green manure (Canali et al. 2015; Watson et al. 2002). Cover crops as green manure crops are particularly important when used as a sustainable fertilization management tool to close gaps in nutrient cycles and to decrease the dependency of external fertilizer purchases. Sown in autumn, they protect against leaching of nitrate (NO_3^-) and other nutrients, reduce wind and water erosion, improve biological, chemical and physical soil properties and store nutrients (Hartwig and Ammon 2002; Fageria et al. 2005; Larkin 2020). Vegetables planted in a previously cultivated leguminous green manure showed advantages over being planted in non-leguminous plants, e.g. velvet bean followed by cabbage (Cordeiro et al. 2018). Therefore, a more targeted approach in organic horticulture could be used to design crop rotations with a higher proportion of legumes to make use of their ability to supply N exclusively via BNF, as N is the nutrient that most often limits yield (Oelofse et al. 2013; Løes et al. 2017; Möller 2018). When cover crops or their residues are tilled and incorporated into the soil in spring, nutrients uptake during their cultivation are released by mineralization and serve as a nutrient source for the subsequent crops. The net N mineralization is mainly dependent on the interactions of soil water content and the C/N ratio of the residues as well as of the soil temperature (Coppens et al. 2007). Farmers can use a wide range of cover crops which are suitable for different purposes. Legumes, in particular, are cultivated for their contribution to the N input into the soil via BNF, thus offering the potential to reduce the need for N fertilizers for the succeeding crop. Nevertheless, some vegetables like cabbage, zucchini or broccoli have very high N demands. Early white cabbage produced for the fresh market has a N requirement of about 200 kg N ha⁻¹, while a slow-growing late white cabbage can take up about 300 kg N ha⁻¹ (Feller et al. 2011). By using legumes as cover crops the amount of N applied as farmyard manure or commercial fertilizer could be reduced and instead be generated internally on-farm.

The objective of this study was therefore to compare the N supply of cover crops by the cultivation of non-legumes, legumes, and the mixture of both crop types used as green manure followed by a late white

cabbage as a high N demanding vegetable crop on its yield in the moderately continental climate of South-West Germany. We hypothesize that (1) legumes as green manures lead to higher cabbage yields compared to non-leguminous green manure or a mixture of both due to a higher N supply via the incorporation of their biomass, (2) a cereal as non-leguminous cover crop leads to a strong decrease of $N_{\min}$ in the soil before the cabbage planting resulting in pre-emptive competition and a reduced cabbage yield, and (3) soil $N_{\min}$ by the time of cabbage harvest and N content in cabbage biomass is increased by the incorporation of leguminous winter cover crops compared to bare soil during winter.

Materials and methods

Study areas and experimental setup

Seven field trials were conducted between 2019 and 2021 on three different locations in southern and western Germany, respectively: at the experimental station Kleinhohenheim (KH, 48°44′14.8″N 9°12′05.6″E) of the University of Hohenheim near Stuttgart; the research field Forchheim, Kaiserstuhl (FH, 48°10′10.3″N 7°42′18.1″E) of the Agricultural Technology Center Augustenberg, both in southwest Germany. The third location was the Experimental Center Horticulture Straelen/Köln-Auweiler (KA, 51°00′04.6″N 6°50′54.0″E) of the North Rhine-Westphalia Chamber of Agriculture in Western Germany. The fields have been organically managed since 1994, 2011 and 1991, the soil types were Haplic Luvisol, Calcaric Regosol and Haplic Luvisol (main soil characteristics are reported in Supplementary Table 1) and the altitudes were 444, 170 and 46 m a.s.l., respectively for KH, FH and KA. The long-term average of the mean annual temperature was 9.7, 11.8 and 11.0 °C and the long-term average of the mean annual precipitation was 740, 670 and 750 mm, for KH, FH and KA respectively. The monthly precipitations and air temperatures are presented in Supplementary Fig. 1. Trials were conducted in each year and location except for 2021 in FH and 2019 in KA. The trials were randomized as randomized complete block design (KA) or as row-column design (KH, FH) with four replicates per trial.

The winter cover crop treatments (I) forage rye (*Secale cereale* L. cv. ‘Protector’) as non-legume, (II) mixture of forage rye with 20% winter Hungarian vetch (*Vicia pannonica* Crantz cv. ‘Beta’) as a mixture of a non-legume with a legume, (III) sole cropped winter Hungarian vetch, (IV) winter pea (*Pisum sativum* L. cv. ‘Leguan’ and ‘EFB 33’), and (V) winter faba bean (*Vicia faba* L. cv. ‘Hiverna’ and ‘Augusta’) were compared with (VI) a control of bare soil during winter and until cabbage planting. In each location, five of the six treatments were tested. Thus, sole cropped winter Hungarian vetch was not tested in KH, winter faba bean was not tested in FH and the mixture of forage rye with 20% winter Hungarian vetch was not tested in KA. The design across locations is therefore similar to an incomplete block design, where each location serves as incomplete block. In total, 55% of all possible treatment-by-year-by-location combinations were available in the data. The imbalance of the dataset was based on two missing trials and one missing treatment per trial. In both cases, the missing data pattern is missing completely at random (Little and Rubin 2019; Hartung and Piepho 2021).

Agronomic measures

Trials were established on different fields at each location due to crop rotation at the location and the specification that the previous crop should be a cereal. The straw from the grain harvest of the preceding cereal was removed and only stubble remained on the fields. Field preparation and seeding of the cover crops included ploughing, disking and preparing the beds in autumn (October to November) depending on local weather conditions and following best agricultural practice at the location. The aim was for the control to be free of weeds. In order not to interfere too much with mineralization processes, weeds were flamed once in spring depending on their occurrence instead of using mechanical weed control. The quantity of weed biomass that grew up despite the control measures was recorded. The cover crops were mulched and incorporated by rototilling as green manure in mid-May. The cash crop was a late maturing cultivar of white cabbage (*Brassica oleracea* convar. *capitata* var. *alba* L. cv. ‘Rivera’). It was grown between the end of May or begin of June and mid of

October. Exact dates of the management measures and trial metrics are listed in Supplementary Table 2.

To secure a sufficient N fertilization level in the cabbage crops, 150 kg N ha⁻¹ of horn shavings was used as a supplement underfoot fertilizer (KH, FH) or via wide spreading on the soil (KA) before planting. This amount was reduced compared to the N requirement of white cabbage (Feller et al. 2011). During the cabbage growing period, weed control was done with a rotary hoe between the rows and by hand within the rows. The trials were covered by a net to protect against pests. Nets were used for the first few weeks at KH, until a few weeks before the cabbage harvest at FH, and for the whole growing period of the cabbage at KA, depending on pest pressure and local practices. During the cabbage cultivation, the products XenTari® (active ingredient: *Bacillus thuringiensis* subsp. *aizawai*, Biofa AG, Germany) against *Lepidoptera* larvae and the product Spruzit Schädlingsfrei (active ingredients: pyrethrin and cinerin, W. Neudorff GmbH KG, Germany) against aphids and other insect larvae were applied if necessary.

Data collection

Soil samples for N_{min} content were taken on six dates at each location and each year. For the assessment of the initial N_{min} content (D1) the soils were sampled in October (0–0.9 m) before establishment of the trials. The number of samples varied depending on the location: In KA and KH, samples for the D1 N_{min} content were taken once per replicate. In FH, only a single sample was taken for the whole trial. In KH in 2021, samples for the D1 N_{min} content were taken from each plot. Subsequent sampling was carried out in all trials on each plot during the complete vegetation period: samples were taken at the beginning of the vegetation period in March (D2; 0–0.9 m), before planting the cabbage in May (D3; 0–0.6 m), at time of the onset of head formation in July (D4; 0–0.6 m), and shortly after the harvest of the cabbage in October (D5, 0–0.6 m). Final samples were taken approximately four weeks after the harvest at the end of the vegetation period in November (D6, 0–0.9 m). As leaching of NO₃⁻ due to precipitation is expected to be highest in Germany at dates D1, D2 and D6, soil sampling was carried out at 0.9 m depth on these dates. In each plot, six soil samples were taken with a

hydraulic soil sampling device and each sample was separated into three sections of 0–0.3 m, 0.3–0.6 m, and 0.6–0.9 m, respectively. Afterwards, the six sub-samples of each section and plot were mixed prior to analysis. Mixed samples were frozen until analysis of the parameters nitrate-N (NO₃⁻-N) and ammonium-N (NH₄⁺-N) via continuous-flow analyzer. The minimum detectable quantification limit for the analysis was 4.5 kg ha⁻¹ for the analyzer used in the laboratory for KH and FH and 2.0 kg ha⁻¹ for the analyzer used in the laboratory for KA for each, NO₃⁻-N and NH₄⁺-N. Values below the detectable limit were arbitrarily set to 50% of the detectable limit, respectively. Finally, the N_{min} content was defined as the sum of NO₃⁻-N and NH₄⁺-N.

During the growing period and before tilling of the winter cover crops, biomass growth was assessed at 0.5 m² per plot at four dates in intervals of approx. two weeks (end of March, mid of April, end of April and mid of May). At the last date, biomass growth of the spontaneous vegetation in the control plots was assessed in addition. The biomass was weighed, dried at 40 °C (KH, FH) resp. 70 °C (KA), weighed again, milled and analyzed for carbon (C) and N concentration by dry combustion. Cash crop yields were assessed as total cabbage yield, head yield (marketable > 1.0 kg, non-marketable < 1.0 kg), and cabbage residue biomass. The fresh and dry matter (DM) as well as the nutrient concentration of C and N of cabbage heads and residues were analyzed also by dry combustion.

The quantity of fixed N via BNF was estimated based on the method of Stuelpnagel (1982) and Karpenstein-Machan and Stuelpnagel (2000). This method requires a non-leguminous reference crop in the trial, and both, plant N and soil N_{min} samples, must be taken in the reference plots. It is therefore a comparatively inexpensive method and easy to perform. In the extended difference method, the plant N and soil N_{min} of the leguminous species are compared to that of a neighbouring non-leguminous species, with the difference between the two treatments assumed to be caused by the N₂ fixation. Accordingly, the sum of the N content in the biomass DM and the soil N_{min} content (total soil profile) of a legume crop was averaged over the trial for each legume containing treatment and subsequently subtracted by the quantity of N content in the biomass DM and the soil

$N_{\min}$ content (total soil profile) of the non-legume reference rye. The differences to the rye are interpreted as quantity of fixed N.

Statistical analysis

All traits were analyzed with a mixed model approach, as mixed models can handle unbalanced data structure with a missing at random or missing completely at random data pattern (Hartung and Piepho 2021). Winter cover crop was used as a fixed factor and year (Y) and location (L) were used as random factors. Our aim was to predict treatment effects in future farmers' fields, therefore we assumed that year and location effects were random (Smith et al. 2005; Piepho et al. 2008). The trials were assumed to represent the population of all possible location-by-year combinations in which cover crops can be used. Additionally, in each location five out of six cover crops were treated. As treatments were not selected according to the expected performance in these locations, this results in a missing completely random data pattern. For soil sample data, a separate analysis for each depth and date was performed. For other samples, a separate analysis was performed for each date. In all cases, the model can be described as (1):

$$y_{hijkl} = \mu + a_h + l_i + (al)_{hi} + r_{hij} + c_{hil} + \tau_k + (\tau a)_{hk} + (\tau l)_{ik} + (\tau al)_{hik} + e_{hijkl} \quad (1)$$

where y_{hijkl} is the observation of k th winter cover crop in column l , replicate j at year h and location i , μ is the intercept, a_h , l_i , $(al)_{hi}$, and r_{hij} are the fixed effects of the h th year, i th location, and h ith year-by-location combination and the fixed effect of the j th replicate nested within a combination of year h and location i . The term c_{hil} is the random column effect. As a randomized complete block design was used in KA, it is blocked and not considered in KA using a dummy variable being 0 for KA and being 1 otherwise (Piepho et al. 2004). The term τ_k is the fixed main effect of the k th winter cover crop, and $(\tau a)_{hk}$, $(\tau l)_{ik}$, and $(\tau al)_{hik}$ are the random interaction effects of the k th winter cover crop with year h , location i and h ith year-by-location combination. The term e_{hijkl} is the error of y_{hijkl} . The model was allowed to account for heterogeneous year-by-location-specific error variances if this increased model fit measured via AIC (Wolfinger 1993). Interaction effects of treatment with year,

location and location-by-year were taken as random to allow a prediction of cover crop performance for other locations and future years (Smith et al. 2005) and to recover inter-location and inter-year information (Möhring et al. 2015). Residuals were graphically checked for normality and homogeneity of variance (despite the heterogeneity already accounted for by the model). These prerequisites were not fulfilled for the trait of soil $N_{\min}$ content; therefore, logarithmic data transformation of the original values was carried out prior to analysis. For the transformed data, estimated means were back-transformed for presentation purpose only. Standard errors were back-transformed using the delta method. In case of finding significant differences via global F test, Fishers LSD test was performed and mean comparisons were presented via letter display (Piepho 2004), accepting a Type 1 error rate of 0.05. Within the letter display, treatment means with at least one identical letter were non-significant different from each other. All analyses were performed with SAS (Statistical Analysis Systems ver. 9.4, SAS Institute, Cary, NC).

Results

Soil mineral N content

The initial soil mineral N ($N_{\min}$) levels for D1 were 90, 28 and 53 kg ha⁻¹ for KH in 2018, 2019 and 2020, 86 and 55 kg ha⁻¹ for FH in 2018 and 2019 and 147 and 161 kg ha⁻¹ for KA in 2019 and 2020.

The $N_{\min}$ content at the beginning of the vegetation period in March (D2) showed significant differences in the depths 0–0.3 m and 0.3–0.6 m (Table 1). The rye treatment had the lowest content at both depths with 8.47 and 9.35 kg ha⁻¹, respectively, and was significantly lower compared to the control, vetch, pea and faba bean (0.3–0.6 m). At the time of the winter cover crop tilling and planting of cabbage (D3), differences were only detected at the depth of 0.3–0.6 m. Again, the treatments rye and rye with vetch showed the lowest $N_{\min}$ contents, while the legume treatments showed no differences when compared to the control. At the time of the onset of head formation of cabbage (D4), the treatments of legumes vetch, pea and faba bean had the highest $N_{\min}$ contents in the soil, at the depth of 0.3–0.6 m significantly with 14.9, 16.2, and 16.5 kg ha⁻¹, respectively, compared to the

Table 1 Average soil $N_{\min}$ content (kg N ha^{-1}) during the winter cover crop and cabbage cultivation period across locations and years between March and November (D2–D6) in the depths 0–0.3, 0.3–0.6, and 0.6–0.9 m

	Start of vegetation	Cabbage planting	Head formation onset	Cabbage harvest	End of vegetation
Winter cover crop	D2	D3	D4	D5	D6
<i>Soil depth 0–0.3 m</i>					
Control	16.7 ± 1.89 ^a	13.2 ± 2.41	17.8 ± 1.98	10.4 ± 0.90	11.8 ± 1.23
Rye	8.47 ± 0.96 ^c	6.61 ± 1.20	15.7 ± 1.68	9.93 ± 0.85	11.6 ± 1.15
Rye + vetch	9.48 ± 1.16 ^{bc}	8.24 ± 1.81	15.7 ± 1.77	9.84 ± 0.92	12.3 ± 1.32
Vetch	15.4 ± 2.14 ^a	16.4 ± 4.01	19.0 ± 2.25	11.1 ± 1.23	13.0 ± 1.58
Pea	13.8 ± 1.56 ^{ab}	15.2 ± 2.79	19.0 ± 2.10	9.79 ± 0.87	12.6 ± 1.32
Faba bean	12.6 ± 1.66 ^{ac}	14.9 ± 3.35	18.6 ± 2.08	11.7 ± 1.04	13.8 ± 1.50
<i>Soil depth 0.3–0.6 m</i>					
Control	22.5 ± 3.37 ^a	12.4 ± 2.05 ^a	13.5 ± 1.73 ^{ab}	5.23 ± 0.48	7.93 ± 0.48
Rye	9.35 ± 1.39 ^c	5.94 ± 0.99 ^c	9.94 ± 1.29 ^{bc}	5.59 ± 0.52	8.20 ± 0.52
Rye + vetch	11.7 ± 1.98 ^{bc}	6.22 ± 1.24 ^{bc}	8.86 ± 1.17 ^c	4.44 ± 0.45	8.49 ± 0.56
Vetch	19.2 ± 3.48 ^{ab}	13.1 ± 2.86 ^a	14.9 ± 2.14 ^a	5.49 ± 0.83	8.15 ± 0.64
Pea	18.4 ± 2.74 ^{ab}	13.1 ± 2.20 ^a	16.2 ± 2.11 ^a	5.09 ± 0.48	8.59 ± 0.54
Faba bean	17.7 ± 3.09 ^{ab}	11.3 ± 2.37 ^{ab}	16.5 ± 2.30 ^a	4.95 ± 0.50	10.3 ± 0.77
<i>Soil depth 0.6–0.9 m</i>					
Control	22.5 ± 2.05	15.3 ± 3.76			3.53 ± 0.47
Rye	15.3 ± 1.45	4.94 ± 1.22			4.21 ± 0.55
Rye + vetch	16.9 ± 1.63	4.89 ± 1.43			4.55 ± 0.73
Vetch	19.4 ± 2.52	13.1 ± 4.07			4.77 ± 0.71
Pea	20.7 ± 1.92	13.5 ± 3.34			5.07 ± 0.69
Faba bean	21.0 ± 2.17	13.9 ± 3.80			4.46 ± 0.80

Values with at least one identical letter indicate non-significant differences among winter cover crop treatments at $\alpha = 0.05$

treatments of rye (9.94 kg ha^{-1}) and rye with vetch (8.86 kg ha^{-1}). As the growing period proceeded, the $N_{\min}$ content continued to decrease across all treatments, so that by the time of the cabbage harvest (D5), the $N_{\min}$ content was almost depleted, especially in the depth of 0.3–0.6 m and no differences between the treatments could be observed. Four weeks after the cabbage harvest and after the incorporation of the cabbage residues at the end of the vegetation period (D6), there were still no differences detected among the treatments.

Cover crop biomass

Biomass DM yield of the cover crops showed no significant differences among treatments at any dates (Table 2), however, rye and rye with vetch consistently showed the highest biomass yields. In comparison, the N concentration of the biomass showed

a significant difference among the three legume treatments with more than 3.0% N in the DM and the treatments rye, rye with vetch or the spontaneous vegetation of the control with 2.0% N in the DM for the last sampling date mid of May. This resulted in rye and rye with vetch having the highest C/N ratios which were significantly higher than the C/N ratios of the three legumes vetch, pea and faba bean or the spontaneous vegetation of the control. The biomass DM yield and N concentration of the different treatments levelled out as N offtake in biomass, whereby no significant differences among the treatments were measured for the sampling dates. The factor year was significant for almost every biomass trait at all four sampling dates whereas the factor location shows less significant differences (Table 3). The estimated BNF of the legume treatments differ non-significantly for rye with vetch (25.9 kg ha^{-1}), vetch (126.1 kg ha^{-1}), pea (117.8 kg ha^{-1}), and faba bean (90.2 kg ha^{-1}).

Table 2 Dry matter (DM) yield, N concentration, C/N ratio and N offtake of the winter cover crop aboveground biomass between end of March and mid of May (shortly before cabbage planting) for all locations and years

Sampling date	Winter cover crop	DM yield (Mg ha ⁻¹)	N concentration (% DM)	C/N ratio	N offtake in biomass (kg N ha ⁻¹)
End of March	Rye	1.79 ± 0.32	2.07 ± 0.22 ^b	21.0 ± 1.55 ^a	32.7 ± 8.72
	Rye with vetch	1.74 ± 0.40	2.36 ± 0.25 ^b	18.4 ± 1.89 ^{ab}	36.3 ± 11.0
	Vetch	0.50 ± 0.43	3.59 ± 0.27 ^a	9.60 ± 2.01 ^c	28.5 ± 11.5
	Pea	0.84 ± 0.32	3.89 ± 0.22 ^a	10.7 ± 1.55 ^c	36.0 ± 8.72
	Faba bean	1.21 ± 0.40	3.26 ± 0.26 ^a	12.5 ± 1.90 ^{bc}	37.2 ± 11.0
Mid of April	Rye	3.06 ± 0.56	1.70 ± 0.14 ^b	26.3 ± 2.33 ^a	52.0 ± 18.4
	Rye with vetch	3.52 ± 0.69	1.99 ± 0.16 ^b	21.8 ± 2.76 ^a	71.3 ± 22.8
	Vetch	0.97 ± 0.72	3.45 ± 0.17 ^a	10.7 ± 2.90 ^b	37.8 ± 23.7
	Pea	1.57 ± 0.56	3.75 ± 0.14 ^a	11.0 ± 2.33 ^b	59.8 ± 18.4
	Faba bean	2.05 ± 0.69	3.42 ± 0.16 ^a	11.9 ± 2.75 ^b	65.2 ± 22.9
End of April	Rye	5.22 ± 0.82	1.36 ± 0.13 ^b	33.8 ± 2.51 ^a	73.7 ± 18.0
	Rye with vetch	5.58 ± 0.92	1.61 ± 0.15 ^b	29.7 ± 3.01 ^a	89.3 ± 21.5
	Vetch	1.87 ± 0.95	3.41 ± 0.17 ^a	9.49 ± 3.32 ^b	76.0 ± 22.5
	Pea	2.61 ± 0.82	3.77 ± 0.13 ^a	11.7 ± 2.51 ^b	100.4 ± 18.0
	Faba bean	3.66 ± 0.93	3.50 ± 0.16 ^a	12.6 ± 3.05 ^b	105.9 ± 22.4
Mid of May	Control	3.27 ± 1.69	2.00 ± 0.27 ^b	19.8 ± 4.14 ^b	56.2 ± 48.3
	Rye	7.77 ± 1.27	1.08 ± 0.19 ^c	42.7 ± 2.84 ^a	85.2 ± 34.7
	Rye with vetch	8.42 ± 1.53	1.23 ± 0.21 ^{bc}	40.8 ± 3.30 ^a	112.5 ± 42.9
	Vetch	5.38 ± 1.61	3.31 ± 0.23 ^a	10.3 ± 3.55 ^b	184.9 ± 45.1
	Pea	5.66 ± 1.27	3.34 ± 0.19 ^a	13.4 ± 2.84 ^b	177.2 ± 34.7
	Faba bean	5.54 ± 1.50	3.21 ± 0.21 ^a	15.5 ± 3.29 ^b	159.2 ± 43.5

Values with at least one identical letter indicate non-significant differences among winter cover crop treatments at $\alpha = 0.05$

Cabbage yield

Due to the high standard errors for the yields of marketable and non-marketable cabbage heads, no significant differences were found among the winter cover crop treatments (Table 4). Nonetheless, the head yield showed a pattern of higher marketable head yields for the legume treatments faba bean (28.0 Mg ha⁻¹), pea (27.3 Mg ha⁻¹) and vetch (25.4 Mg ha⁻¹) followed by the control (21.4 Mg ha⁻¹), rye with vetch (14.6 Mg ha⁻¹), and rye (11.3 Mg ha⁻¹). There were differences in the fresh mass yield of marketable heads among the trials (Table 3). For the residue DM, non-significant differences were found among the treatments, but were found among trials. The same was observed for the total above ground DM biomass (for treatments $p = 0.1446$) (Table 3).

Cabbage N content

The N concentrations in the cabbage heads were higher compared to the concentrations of the cabbage residues for all treatments (Fig. 1). The N concentration in total above ground biomass (heads and residues) was significantly lower in the treatment with rye compared to the control, vetch, pea and faba bean, and in vetch with rye compared to vetch and pea. No differences were detected between the control and the leguminous treatments.

The N offtake by the cabbage heads was significantly lower in rye and rye with vetch compared to all other treatments (Fig. 2). The same pattern was observed in the offtake of the total above ground biomass. Nonetheless, the N offtake for the residues did not differ significantly among the treatments. Again, the traits above ground biomass, cabbage heads and cabbage residues showed differences in the factors of year and interaction of year and location (Table 3).

Table 3 Overview of the sources of variation and probability (*P*) values, which correspond to global F tests from the analysis of variance

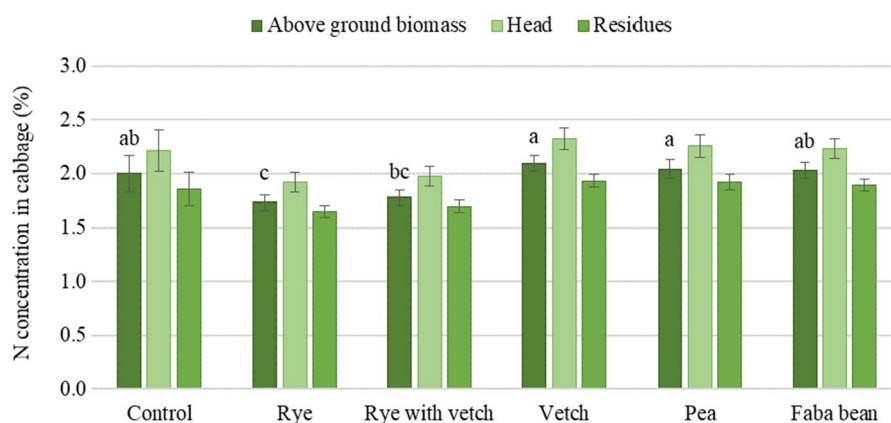
Source of variation	<i>P</i> values ^a											
Soil N _{min} content (Table 3)												
Date	D2			D3			D4			D5		D6
Depth (m)	0–0.3	0.3–0.6	0.6–0.9	0–0.3	0.3–0.6	0.6–0.9	0–0.3	0.3–0.6	0–0.3	0.3–0.6	0–0.3	0.3–0.6
Winter cover crop	0.0314	0.0292	0.0651	0.0609	0.0395	0.0628	0.2397	0.0282	0.6593	0.4858	0.4384	0.1605
Year (Y)	0.4681	0.6313	0.0448	0.0012	<.0001	0.0061	0.0003	0.8414	<.0001	0.7608	0.0819	0.0002
Location (L)	0.0215	0.0052	<.0001	0.0044	<.0001	0.0011	0.0228	<.0001	0.1251	<.0001	0.3922	0.3157
Y × L	0.0357	0.0004	<.0001	0.0128	<.0001	0.0004	0.2504	0.1282	0.0043	^{2b)}	0.3654	<.0001
Winter cover crop biomass (Table 1)												
End of March			Mid of April			End of April			Mid of May			
Dry mass	N conc.	C/N ratio	N offtake	Dry mass	N conc.	C/N ratio	N offtake	Dry mass	N conc.	C/N ratio	N offtake	N offtake ratio
Winter cover crop	0.2055	0.0017	0.0126	0.9743	0.1513	0.0003	0.0088	0.8584	0.0628	<.0001	0.0062	0.7350
Year (Y)	0.0073	0.1163	0.0332	<.0001	0.0151	0.0053	0.0390	0.0017	0.0096	0.0031	0.1305	0.0010
Location (L)	0.1252	0.0690	0.4417	0.1252	0.0928	0.0125	0.3652	0.1073	0.1081	0.0276	0.2094	0.0493
Y × L	0.0336	0.3512	0.1509	0.0008	0.0052	0.0801	0.2708	0.0060	0.0750	0.0251	0.1162	0.5201
Biological N ₂ fixation												
Cabbage yield (Table 4)												
N concentration (Fig. 1)												
N offtake (Fig. 2)												
Before cabbage planting	Head yield (> 1.0 kg)	Head yield marketable (> 1.0 kg)	Head yield non-marketable (< 1.0 kg)	Residues DM bio-mass	Above ground biomass	Above ground DM biomass	Above ground biomass	Cabbage heads	Cabbage residues	Above ground biomass	Cabbage heads	Cabbage residues
Winter cover crop	0.0872	0.1446	0.6560	0.1674	0.1446	0.0428	0.0814	0.0661	0.0100	0.0019	0.3833	
Year (Y)	<.0001	<.0001	0.4240	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001
Location (L)	0.1443	0.0025	0.1211	<.0001	0.0025	0.0002	0.0001	0.0005	0.9615	<.0001	0.0654	
Y × L	0.3465	0.0002	0.3806	0.0006	0.0002	<.0001	<.0001	<.0001	<.0001	0.0024	0.0001	

^a*P* values correspond to global F tests of differences between levels of the corresponding factor from the analysis of variance^bData sets of the same location, date and depth with all samples below the detection limit were removed from the analysis; resulting in no estimable interaction of year and location (Y × L) when only one year of one location left

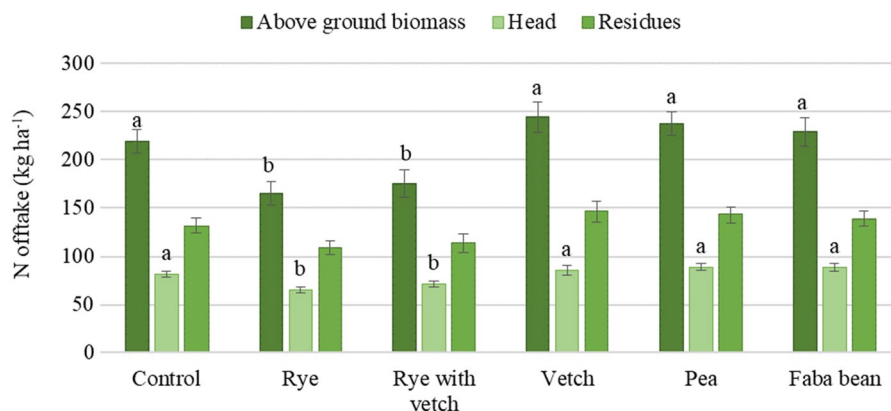
Table 4 Influence of winter cover crops on cabbage head fresh matter yield (Mg ha^{-1}), indicated as marketable yield (heads > 1.0 kg) and non-marketable yield (heads < 1.0 kg) as well as DM biomass of cabbage residues for all locations and years

Cover crop	Marketable heads (> 1.0 kg) (FM) Mg ha^{-1}	Non-marketable heads (< 1.0 kg) (FM) Mg ha^{-1}	Residues (DM) Mg ha^{-1}	Total above ground biomass (DM) Mg ha^{-1}
Control	21.4 ± 3.43	15.8 ± 2.45	6.98 ± 0.26	11.5 ± 0.45
Rye	11.3 ± 3.43	19.8 ± 2.45	6.41 ± 0.23	10.1 ± 0.40
Rye + vetch	14.6 ± 3.96	18.9 ± 3.04	6.71 ± 0.30	10.9 ± 0.40
Vetch	25.4 ± 4.58	14.2 ± 3.48	7.05 ± 0.33	10.7 ± 0.47
Pea	27.3 ± 3.48	14.5 ± 3.52	7.49 ± 0.28	11.8 ± 0.41
Faba bean	28.9 ± 4.40	13.8 ± 2.87	7.20 ± 0.31	11.4 ± 0.49

Values with at least one identical letter indicate non-significant differences among winter cover crop treatments at $\alpha = 0.05$

**Fig. 1** Influence of winter cover crop on N concentration of cabbage heads and residues after harvest for all locations and years. The results are the means of four replicates and the error bars show the standard error. Values with at least one identical

letter within the plant part (above ground biomass, heads, residues) indicate non-significant differences among winter cover crop treatments at $\alpha = 0.05$

Fig. 2 Influence of winter cover crop on N offtake by harvested cabbage heads and remaining residues after harvest for all locations and years. The results are the means of four replicates and the error bars show the standard error. Values with at least one identical letter indicate non-significant differences among the treatments at $\alpha = 0.05$ within each plant part

Discussion

N supply of leguminous and non-leguminous green manures to white cabbage

Although the cabbage yield did not differ significantly among the treatments (Table 4), the data on cabbage above ground biomass N concentration (Fig. 1) and cabbage above ground biomass N offtakes (Fig. 2) show that legumes used as winter cover crops and subsequently as green manures provide a higher N supply for the subsequent vegetable crop compared to sole cropped rye and the intercropping of rye with vetch. A high N accumulation in the winter cover crops depends on high biomass DM yield and/or high N concentration (Table 2). Therefore, leguminous green manures should be selected on criteria including consistent and strong biomass production potential. Since legumes generally are higher in biomass N concentration due to their ability to fix atmospheric N (Hartwig and Ammon 2002), higher N concentrations were found in vetch, pea and faba bean at the incorporation date in comparison to the non-legume rye and the mixture of non-legume and legume rye with vetch, in line with other studies (Campiglia et al. 2014a, b; Nyfeler et al. 2011).

C/N ratio of late tilled leguminous green manures

Besides the N quantity provided for the subsequent crop, the rate of N release after incorporation plays a crucial role for the farmer's choice of green manure species: lower C/N ratios favour a faster decomposition of the green manure residues incorporated into the soil (Coppens et al. 2007). Generally, net N mineralization can be expected when the C/N ratio is < 20–25, while net immobilization can be expected when the C/N ratio is > 20–25 (Paul 2007). Legumes, therefore, show a higher potential of fast decomposition with C/N ratios between 10 and 15 (Rosecrance et al. 2000; Campiglia et al. 2014a) compared to non-legumes (Table 2).

Data in Table 2 indicates that the nutrient composition of the biomass of legumes changes during the cropping phase of the green manure to much lesser extent than that of non-legumes and the mixtures. Consequently, and assuming that the N concentration and the C/N ratio are the major drivers for the N mineralization pattern after green manure

incorporation, it can be stated that the date of soil incorporation of the green manure biomass is much more relevant for non-legume green manure crops than for legumes. Furthermore, for legumes, there is another feature that should be addressed when determining the incorporation date: a later incorporation is connected to only a slight increase of the C/N ratio, however, combined with a strong increase of the total amount of N incorporated with the biomass, meaning a strong additional BNF. No studies were found addressing specifically the effects of the date of soil incorporation of legumes on BNF and the effects on the N supply of the subsequent crops. The legume cover crops in this study were able to fix an average of 90 kg N ha⁻¹ until mid-May, this corresponds to 37% of the N offtake of organic white cabbage above ground biomass. For less demanding crops than cabbage, the proportion of N offtake covered by BNF from legume winter cover crops can be up to 60% for carrots, or even 117% for lettuce (Wachendorf et al. 2022). However, the accuracy of the BNF estimation is rather low using Stuelpnagel's (1982) difference method compared to the recommended and more accurate measurements of symbiotic N₂ fixation using ¹⁵N labelled legumes (Unkovich et al. 2008).

For the parameters biomass DM yield of the cover crop, N concentration, C/N ratio, and N offtake of biomass, the mixture of rye with the legume vetch does not show significant advantages compared to sole cropped rye. The estimated amount of BNF for rye with vetch is about 26 kg N ha⁻¹. In comparison, the three leguminous treatments vetch, pea and faba bean showed higher values with 126, 118 and 90 kg ha⁻¹, respectively. Therefore, in order to achieve high N inputs by BNF a higher share in the mixture or pure legumes stands should be favoured. Frasier et al. (2017) used the same estimation formula for BNF by Karpenstein-Machan and Stuelpnagel (2000) but a 40/60 mixture of rye with vetch showing similar high values for BNF (100 kg ha⁻¹) compared to sole cropped vetch (110 kg ha⁻¹). Therefore, the treatment of rye with vetch could provide more advantages as a combination of catch crop and green manure instead of sole cropping of rye if the vetch proportion was higher than the 20/80 mixture in our study (Nyfeler et al. 2011).

Pre-emptive competition on soil $N_{\min}$ by non-leguminous cover crops

Besides the N supply provided via green manure biomass, the soil $N_{\min}$ content plays a major role in the development of vegetable plants at early growth stages. A pre-emptive competition for soil $N_{\min}$ prior to planting can lead to yield loss in high demanding vegetable crops (Willumsen and Thorup-Kristensen 2001; Hefner et al. 2020). Especially cereal crops, mainly used as catch crop during winter, show high N uptake which is then initially unavailable to the following crop after incorporation of the winter cover crop due to the high C/N ratio of the biomass resulting in N immobilization. The pre-emptive competition can also be observed in our study (Table 1) for the sampling dates D2 (start of vegetation period) and D3 (prior to planting). After the rainy winter period the treatments of rye and rye with vetch had the lowest soil $N_{\min}$ contents at the start of the vegetation period and at the time of cabbage planting. This could indicate that NO_3^- leaching was prevented during winter by the strong uptake of the cereals or it also could indicate that NO_3^- leaching occurred and could therefore not be measured in the lowest soil layer. The first case appears more likely due to the high dry matter already produced in March and the higher N concentrations in rye and rye with vetch compared to the end of the cropping period (Table 2). If the N was leached as NO_3^- , both dry matter and N concentration would likely be lower. The legumes, in contrast, did not significantly lower the $N_{\min}$ content during these periods compared to the control. As catch crops for reduction of nitrate leaching the legumes are therefore less suitable. The soil $N_{\min}$ content at the time of cabbage head formation onset (Table 1, D4), however, supports the assumption of rapid N release and thus green manuring with legumes resulting in the highest $N_{\min}$ values among cover crop treatments in both depths, 0–0.3 and 0.3–0.6 m (Haas et al. 2007; Campiglia et al. 2014b).

At the end of the cabbage cultivation and the vegetation period itself (D5 and D6) no differences among the different cover crop treatments were observed. The high N demanding cabbage crop depleted the available soil $N_{\min}$ in all treatments. However, there are some indications for N shortage in the cabbage for the treatments with non-leguminous crops as stated by lower cabbage N concentrations and N offtakes in

the treatments with rye and rye with vetch as preceding crop (Figs. 1 and 2). The lower cabbage N offtake for the treatments rye and rye with vetch – in case of heads even significantly lower – compared to the other treatments (Fig. 2) seems primarily based on differences in N concentration rather than on the residue DM yields (Table 3).

Comparing leguminous green manuring with no preceding crop in the cabbage cultivation

Contrary to the initial hypothesis, the result of this study showed no significant increase in cabbage head yield with green manuring via leguminous cover crop species before planting compared to the bare soil during winter (Table 4). This lack of differences can be explained to some extent by the high standard errors for both, the marketable and the non-marketable head yield. Different initial $N_{\min}$ contents at the time of the trial establishment may have had an impact on the cover crops development and therefore on the biomass and N offtake of the green manure or the amount of fixed N_2 . However, a correlation between initial soil $N_{\min}$ content and cover crop development or amount of BNF could not be investigated in this work because soil samples were not taken per plot when the trials were established. Another explanation for the lack of significant differences between bare soil and leguminous winter cover crops could be the spontaneous vegetation, which was allowed to grow to a reasonable extent in order not to interfere too much with the mineralization processes of the soil by mechanical tillage. At the time of incorporation of the winter cover crops, weed biomass had grown on these plots, possibly resulting in a green manuring effect as well. In agricultural practice, such spontaneous vegetation would probably be mechanically removed earlier.

Overall, the cabbage head yield –either total head yield or marketable head yield– in our study is in line with results of similar studies including leguminous green manures followed by white cabbage like Haas et al. (2007) or Hefner et al. (2020) with between 20 and 50 Mg ha⁻¹. Considering the reduced amount of fertilizer with 150 kg N ha⁻¹ in this study compared to the N demand by cabbage of at least 200 kg N ha⁻¹, it can be assumed that the leguminous green manure was not capable of overcompensating the fertilizer reduction within

the growing period of the cabbage and of leading to significantly increased cabbage yields. An effect of incorporated biomass of green manures - especially legumes and their BNF - compared to bare soil over winter, however, could be detected in the subsequent crop to cabbage. A systemic investigation of the crop rotation is therefore recommended.

Conclusion

As external N fertilizer inputs are often considered contentious when derived from conventional farming systems, the use of N₂ fixing leguminous winter cover crops such as vetch, pea and faba bean as green manures is an option to substitute them. However, the amount of N supplied by leguminous cover crops does not fully substitute commercial keratin-based fertilizers for a vegetable cropping system with high N demanding crops like cabbage. Nevertheless, legumes are well suited as preceding green manure crops for vegetables, as they contain high levels of N in their biomass and do not pre-emptively compete with the following crop for the soil mineral nitrogen. The N in the biomass is readily available because of the narrow C/N ratio, resulting in rapid mineralization in the soil. By increasing the proportion of vetch in the mixture with rye, the combination of these two species could be useful for the reduction of NO₃⁻ leaching over winter while providing high amounts of N for the subsequent high N demanding vegetables in late spring. The main result of this study and benefit of pure legume stands as green manure crops is their low increase in the aboveground biomass C/N ratio over a period of several weeks in spring. To our knowledge, no data is available on how the change in N amount and biomass composition over the entire spring period influences the N mineralization pattern and the N release from winter cover crop residues after soil incorporation. Therefore, this should be investigated in the future, as well as the influence of leguminous winter cover crops on the succeeding crops in the crop rotation.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare no financial and non-financial conflicts of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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