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Article

The Effects of Leguminous Living Mulch Intercropping and Its Growth Management on Organic Cabbage Yield and Biological Nitrogen Fixation

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Abstract: In organic horticulture, living mulches (LM) are used for weed suppression and erosion prevention. In addition, leguminous LM can contribute to higher nitrogen (N) import into vegetable cultivation systems via biological N_2 fixation (BNF). In order to investigate the effect of LM systems, a two- as well as three-year field experiment was conducted between 2019 and 2021 at two locations in Southwest Germany. White cabbage was intercropped with two different clover varieties (*Trifolium repens* cv. 'Rivendel', with regular growth and *T. repens* cv. 'Pipolina', a micro clover) and perennial ryegrass (*Lolium perenne* cv. 'Premium'). Bare soil (with spontaneous vegetation) without intercropping was the control treatment. The second factor was the growth management of the LM: incorporation by rototilling before planting the cabbage, intercropping with the cabbage and no LM growth management, and intercropping with mulching of the LM during the cabbage growing. The results show that rototilling LM before planting the cabbage did not lead to higher weight of cabbage residues or differences in total head yield among the treatments for growth management. Intercropping without further LM growth management did not result in a reduced total head yield of cabbage compared to mulching. The micro clover 'Pipolina' showed no reduced competition with cabbage compared to the regular-growing white clover 'Rivendel'. Therefore, we conclude that leguminous LM systems, regardless of growth management, can achieve high yields with sufficient irrigation and additional fertilization while increasing the inputs of N via BNF into the entire cropping system.

Keywords: horticulture; legumes; N_2 fixation; fertilization; intercropping; vegetables

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1. Introduction

The organic cultivation of vegetables with a high demand for nutrients, especially nitrogen (N), within a comparatively short growing period is challenging. One method of fertilizing vegetables is using farmyard manures, e.g., cattle manure, but this often does not overcome the challenge of a well-timed nutrient release. Furthermore, the nutrient stoichiometry of manure does not match the nutrient offtake by vegetables, and often leads to nutrient imbalances, particularly an oversupply of phosphorus (P) in the soil [1–3]. Additionally, there is often little to no animal husbandry in intensive organic vegetable production. Purchasing external commercial fertilizers is therefore another option; however, this is difficult to reconcile with the conceptual framework of organic farming as it does not comply with the organic principle of closing the on-farm nutrient cycle [4]. Furthermore, external commercial fertilizers often consist of products or by-products of intensive conventional animal husbandry systems, which qualify them as so-called “contentious inputs” that need to be phased out and even, in some cases, require approval from organic associations [5]. For these reasons, the number of fertilizers is already limited in organic farming by the restrictions of different governmental standards, e.g., of the Council Regulation on Organic Food and Farming in Europe [6]. Further restrictions are expected to follow in the next years, e.g., by some standards of private farmers associations for organic farming [7]. In order to meet the national and international increasing demand for organically produced vegetables [8–10] and, at the same time, supply the plants with sufficient amounts of nutrients, an improved, well-balanced fertilization management is required in organic horticulture.

The use of legumes to provide N to the soil via biological N_2 fixation (BNF) is an essential element in organic fertilization strategies. Legumes can be used as sole crops, e.g., as cover crops or perennial leys, fulfilling several functions such as the reduction in soil erosion [11] or nitrate leaching [12]. However, leguminous cover crops can also be intercropped with a main crop serving as living mulches (LM).

Systems of LM are primarily used for their weed suppression effects during the intercropping with the main crop reducing the intensive soil tillage typical for organic horticulture. Such systems are already being studied for suitable combinations of vegetable crops and LM, e.g., cabbage with vetch or broccoli with winter rye [13,14]. Especially in organic farming, reduced soil tillage in combination with cover crops leads to higher contents of soil organic matter [15]. In addition to the beneficial properties of the cover crops mentioned above, further advantages of LM systems include the moderation of fluctuations in soil temperature and a higher water infiltration [16–18]. Brandsæter et al. [19] showed that clover LMs lead to a higher white cabbage yield as compared to cabbage monoculture due to lower pest damage. However, despite these advantages, the competition for light, water and nutrients between the main crop and the LM can affect crop growth in LM systems [20]. In some cases, advantages, e.g., even soil temperatures during the year, might turn out to be disadvantageous. As shown by Borowy [21] in tomato cultivation, lower soil temperatures due to shading of LM resulted in lower fruit yields.

Nevertheless, vegetable production could be particularly suitable for LM systems in terms of competition for light and water: Many vegetable crops are grown in wide rows and are additionally irrigated which minimizes the risk of water stress for the main crop. Due to their BNF ability, legumes are suitable partners in these intercropping systems as competition for nutrients, at least for N, is reduced compared to non N_2 fixing crops. In order to compete with the main crop as little as possible, a lower growth and a low nutrient demand for reduced competition with the main crop is favorable. Hairy Vetch (*Vicia villosa*) and clover varieties are preferred and frequently used as LM due to their ability to suppress weeds and their BNF ability [22]. Several studies already indicated that leguminous LM systems may work in vegetable production, especially with *Brassicacea* as a main crop which has been shown to maintain or increase the yield in cultivation of, for example, broccoli with white clover (*Trifolium repens* L.) [23,24], cauliflower with annual clover (*T. resupinatum* L.) [25], broccoli with red clover (*T. pratense* L.) [26], cauliflower with grass-clover [27] or cauliflower with burr medic (*Medicago polymorpha* L.) [28].

Additional factors of cultivation also play a crucial role in LM systems. Results of previous studies showed that sowing date and growth management of the LM have a decisive influence on yield development of the main crop. Sowing LM after planting the main crop reduces competition; however, a successful establishment of LM in the previous fall could provide soil cover, lower nitrate leaching during winter and lower weed pressure in spring. Additionally, it can be expected that earlier sowing prolongs the growing period of the leguminous LM and thus the amount of N_2 fixation. A further aspect to consider is the growth management of LM during cultivation of the main crop. Early sowing of the LM could lead to stronger competition due to its high biomass at the time of planting the vegetables. To mitigate this competition during the first weeks of the vegetable plantlets development, LM biomass could be cut. In addition, mowing LM biomass reduces weed seed production and thus may also have a long-term impact reducing the soil weed seed bank in organic farming [29].

Since the performance of LM systems is dependent on many factors, Canali et al. and Gruszecki et al. [30,31] recommended further investigation of LM systems for sustainable vegetable production. The goal of further research on LM systems should therefore focus on avoiding yield loss or at least counterbalancing a possible yield reduction in the vegetable main crop while maintaining the additional ecosystem services provided such as increasing biodiversity, preventing erosion and suppressing weeds [30,32]. This requires solutions for reducing the competition within the systems. We address the research question by looking at whether (1) strip tilling of cabbage into a leguminous LM will lead to competition between LM and cabbage during cultivation resulting in a significant yield decline compared to complete incorporation of LM before cabbage planting, (2) cutting leguminous LM in the vegetable row will reduce competition with the cabbage leading to higher yields compared to the untreated LM, and (3) a dense and low-growing leguminous LM such as micro clover exerts less competition on cabbage plants compared to a white clover variety of a regular growth type, therefore resulting in a higher cabbage yield.

2. Materials and Methods

The field experiments were conducted in Southwest Germany at the organic research station Kleinhohenheim of the University of Hohenheim (UH) for three years (2019–2021) and at the organic experimental field site Grötzingen (GR) of the Centre for Agricultural Technology Augustenberg for two years (2019–2020). The altitudes are 435 m a.s.l. (UH) and 120 m a.s.l. (GR). The mean annual precipitations are approx. 740 mm (UH) and 750 mm (GR), and long-term annual average temperatures are 9.7 °C (UH) and 10.1 °C (GR). The monthly temperature and precipitation profiles of the experimental years are shown for both sites in Figure 1. The soil types are a Haplic Luvisol form sandy and loamy substrate with loess (UH) and a Gleysol from loamy to clayey fluviatile sediments (GR) (source: https://maps.lgrb-bw.de/ (accessed on 5 January 2022)).

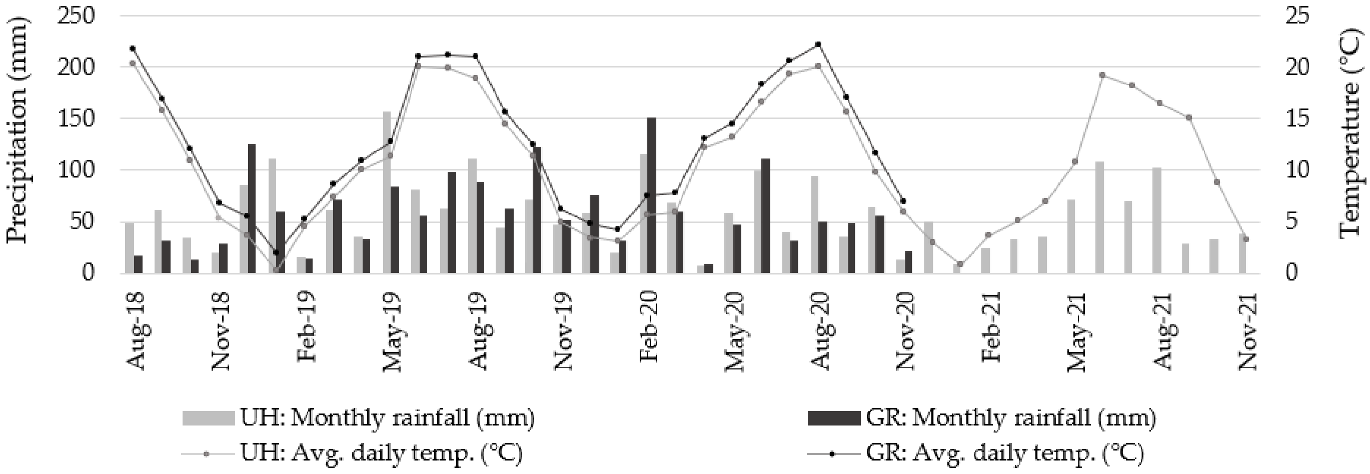

Figure 1. Monthly precipitation (mm) and temperature (°C) of the trial period between August 2018 and November 2021 at Grötzingen (GR) and the University of Hohenheim (UH). Source: www.wetter-bw.de, weather stations Grötzingen and Hohenheim.

The trials were conducted in a two-factorial split-plot design with four replicates (the main-plot factor LM growth management with three levels and a sub-plot factor LM species with four levels). For the factor LM growth management, the levels were (i) complete rototilling (RT) of LM before planting cabbage, (ii) transplanting cabbage via strip tilling in LM with no further treatment of LM (strip till + untreated, STU), and (iii) transplanting cabbage via strip tilling in LM with mowing of the LM during the growing period of the cabbage; (strip till + treated, STT). For the sub-plot factor LM species, the following LMs were used: two white clover varieties *Trifolium repens* cv. (i) ‘Rivendel’ (white clover = WC, vigorous growth/variety for fodder production) and *T. repens* cv. (ii) ‘Pipolina’ (micro clover = MC, reduced height, but dense growth), (iii) perennial ryegrass (=RG) (*Lolium perenne* cv. ‘Premium’) as non-leguminous reference, and (iv) bare soil as control (=C). At the location of UH both clover varieties had to be re-seeded in early April in 2019 and 2020 with a seeding density of 20 kg ha^{-1} due to water stress that resulted in insufficient establishment. This resulted in two further levels of the sub-plot factor with both white clover varieties established in (v) fall (-F) of the previous year and (vi) spring (-S) of the actual trial year (Table 1). Established clover–cabbage and ryegrass–cabbage intercropping is shown in Figure 2A,B.

Table 1. Overview of treatments within main-plot factor growth management of LM and sub-plot factor LM species with abbreviations.

	Factor: Growth Management of LM	Abbr.		Factor: LM Species	Abbr.
(i)	Complete rototillage and incorporation of LM before planting cabbage	RT	(i)	White clover—fall seeded	WC-F
(ii)	Strip-till cabbage planting into LM without further treatment of LM	STU	(ii)	Micro clover—fall seeded	MC-F
(iii)	Strip-till cabbage planting into LM with mulching LM during cabbage growing	STT	(iii)	Perennial ryegrass	RG
			(iv)	Bare soil (=control)	C
			(v)	White clover—spring seeded	WC-S
			(vi)	Micro clover—spring seeded	MC-S

Figure 2. Established clover–cabbage (**A**) and ryegrass–cabbage (**B**) intercropping.

The LM were sown in September of the previous year with seeding rates of 40 kg ha^{-1} for the perennial ryegrass, 20 kg ha^{-1} for white clover cv. ‘Rivendel’, and 10 (2019) as well as 20 kg ha^{-1} (2020 and 2021) for micro clover cv. ‘Pipolina’. For the treatment complete rototilling (RT), the LMs were mulched and incorporated into the soil shortly before cabbage planting. Late white cabbage (*Brassica oleracea* convar. *capitata* var. *alba* cv. ‘Rivera’) as the vegetable main crop was grown between the end of May and mid-October for 18–20 weeks. A total of 150 kg N ha^{-1} via horn grit was used as root dressing to meet the high N demand of late white cabbage of at least 200 kg N ha^{-1} [33]. In both strip till treatments, the cabbage was transplanted into strips with 0.25 m width. Differences between years and locations are displayed in Table 2.

Table 2. Overview of differences in clover establishment, initial mineral nitrogen (N_{min}) content, plot size and spacing of cabbage planting in the trials in both locations and for three years.

Site Differences	2019		2020		2021
Location [1]	UH [1]	GR [1]	UH	GR	UH
Clover establishment	spring	fall	spring	fall	fall
Initial N_{min} content ($kg\ ha^{-1}$ at 0–90 cm)	91	106	38	119	45
Cabbage planting					
Plot size (m^2)	11 × 4.5	11 × 3	11 × 3	11 × 3	11 × 3
Rows per plot	6	4	4	4	4
Inter-row spacing (m)	0.75	0.5	0.75	0.65	0.75
Intra-row spacing (m)	0.32	0.5	0.32	0.5	0.32
Plants ha^{-1}	42,000	40,000	42,000	31,000	42,000

[1] UH = University of Hohenheim, GR = Grötzingen.

For the first six to eight weeks, the trials were covered by a net to protect against pests. When necessary, pesticides approved for use in organic farming were used to control pest and disease pressure. During the growing period of cabbage, weed control in the completely rototilled treatment (RT) was performed by rotary hoe and by hand within the rows of strip till treatments. The control plots within the strip till treatments were managed in the same way as the treatments with LM and therefore were not mechanically tilled. To avoid high biomass growth of spontaneous vegetation, the plots in the control treatment were flamed once before cabbage planting. Subsequently, no weed suppression measure was conducted in the control between the cabbage rows. Irrigation was carried out by sprinkler systems averaging around 80 $L\ m^{-2}\ a^{-1}$ for each location, according to the assessment of the institutions' agricultural technicians, to prevent drought stress of the plants. Winter wheat (*Triticum aestivum* cv. 'KWS Livius') was sown as a subsequent crop to the cabbage for further investigations on the carry-over effect of the LM system (data not shown).

Soil sampling for mineral nitrogen (N_{min}) was performed six times during the experiments. The initial N_{min} contents at a depth of 90 cm (D1) for the individual years and locations are listed in Table 2. The following soil samplings were performed at the start of the vegetation period in March (D2, 0–90 cm), shortly before planting of the cabbage end of May (D3, 0–90 cm), at the time of cabbage head formation onset middle of July (D4, 0–60 cm), at the time of cabbage harvest in October (D5, 0–60 cm) and four weeks after cabbage harvest in November (D6, 0–90 cm).

The soil samples were extracted by $CaCl_2$ and analyzed for nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) using Continuous Flow Analysis (CFA Evolution II, Alliance Instruments, Austria) [33]. The detection threshold of the CFA for the NO_3^--N and NH_4^+-N was 4.5 $kg\ ha^{-1}$. In samples where this limit was not reached, we assumed that the NO_3^--N and NH_4^+-N content was between 0 and 4.5 $kg\ ha^{-1}$; therefore, an arbitrary value of 2.25 $kg\ ha^{-1}$ was used for calculations [34]. In 2019 and 2020, NO_3^--N and NH_4^+-N content was related to a standard soil dry matter (DM) of 80%. In 2021 the dry matter of the soil was measured as well as the respective water content which was related to the actual soil DM. Both parameters, NO_3^--N and NH_4^+-N, were summed up as soil N_{min} content.

The LM biomass was sampled before complete rototilling and incorporation of the LM for the cabbage planting in the RT treatment. Additionally, a second biomass sample was taken for all three years in UH, two weeks before the cabbage harvest and therefore, shortly before the incorporation of the remaining LM (in treatments STU and STT) and cabbage residues. DM of LM, including weeds, was recorded after cutting the aboveground biomass by drying at 40 °C until constant weight. After drying, the plant samples were milled and further analyzed for carbon (C) and N concentration by dry combustion (vario MAX cube, Elementar, Langenselbold, Germany).

The amount of N fixed by legumes was estimated based on the prolonged difference method of Stuelpnagel [35]. This method was used for the first LM biomass sampling in GR for 2019 and in UH for 2020 and 2021 as well as for the second LM biomass sampling in UH for 2019–2021. To obtain estimates for BNF of leguminous LM, the sum of the N content in the biomass and the soil N_{min} content (total soil profile) was calculated. The BNF for white and micro clover was calculated as the difference compared to the reference perennial ryegrass. Soil-plant analysis development measurements (SPAD, SPAD-502Plus, Konica Minolta Sensing Europe B.V., Nieuwegein, Netherlands) of the oldest, non-senescent cabbage leaves (20 plants per plot) were taken twice, at the time of head formation onset and two weeks before harvest. Cabbage yield was assessed 18 to 20 weeks after planting with regard to head yield and residues. Cabbage heads and residues were dried at 40 °C until constant weight and subsequently analyzed for C and N.

All traits were analyzed using a mixed model approach with growth management of LM (main-plot factor) and LM species (sub-plot factor) as fixed factors and using year (Y) and location (L) as random factors. For soil sample data, a separate analysis for each depth was performed. The model can be described as (1):

$$\begin{aligned} y_{hijkl} = \mu + a_h \quad & + l_i + (al)_{hi} + r_{hij} + \tau_k + \theta_l + (\tau\theta)_{kl} + (\tau a)_{hk} + (\theta a)_{hl} \\ & + (\tau\theta a)_{hkl} + (\tau l)_{ik} + (\theta l)_{il} + (\tau\theta l)_{ikl} + (\tau al)_{hik} + (\theta al)_{hil} \\ & + (\tau\theta al)_{hikl} + f_{hijk} + e_{hijkl} \end{aligned} \tag{1}$$

where y_{hijkl} is the observation of kth growth management and lth species in 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 hth year, ith location, and hith year-by-location combination and the fixed effect of the jth replicate nested within a combination of year h and location i. τ_k, θ_l, and $(\tau\theta)_{kl}$ are the main fixed effects of kth LM growth management and lth LM species as well as its interactions. $(\tau\theta)_{kl}$, $(\tau a)_{hk}$, $(\theta a)_{hl}$, $(\tau\theta a)_{hkl}$, $(\tau l)_{ik}$, $(\theta l)_{il}$, $(\tau\theta l)_{ikl}$, $(\tau al)_{hik}$, $(\theta al)_{hil}$ and $(\tau\theta al)_{hikl}$ are the random interaction effects of treatment effects with year, location and year-by-location; f_{hijk} and e_{hijkl} are the main- and sub-plot error from the split-plot design. The model allowed accounting for heterogeneous year-by-location-specific error variances if this increased model fit measured via AIC [36]. Residuals were graphically checked for normality and homogeneity of variance (despite heterogeneity that was already accounted for by the model). If these prerequisites were not fulfilled, data transformation of the original values was carried out prior to analysis: Data of total head yield, C/N ratio of LM biomass (of the first sampling prior to cabbage planting), and all N_{min} values were logarithmically transformed. For the transformed data, the means were back-transformed for purpose of presentation only. In cases where significant differences were found via global F test, Fisher's LSD test was performed and mean comparisons were presented via letter display [37], accepting a Type 1 error rate of 0.05. Within the letter display, means with at least one identical capital letter showed non-significant differences among the main-plot factor level. Means with at least one identical lower-case letter indicated non-significances among treatments of the sub-plot factor. All analyses were performed with SAS (Statistical Analysis Systems ver. 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Soil Mineral Nitrogen (N_{min})

No significant interactions between growth management and LM for the soil N_{min} contents were shown. An overview of the sources of variations and p values which correspond to global F tests from the analysis of variance for each trait is displayed in Table 3. Individual significances within the main plot or sub plot factors are marked in Table 4.

In addition, the significances of the location factor are shown in Table 3. Differences were found for the dates D1, D2 for 0–30 cm, D3 for 30–60 cm, D4 for 30–60 cm, D5 for 0–30 cm and D6 for 60–90 cm.

Table 3. Overview of the sources of variations and *p* values which correspond to global F tests from the analysis of variance.

Source of Variation	***p* Values ***													
	Soil N_{min} content (Table 4)													
Date	D1	D2			D3			D4		D5		D6		
Depth (cm)	0–90	0–30	30–60	60–90	0–30	30–60	60–90	0–30	30–60	0–30	30–60	0–30	30–60	60–90
Living mulches (LM)	-	0.3055	0.0790	0.0735	<0.0001	<0.0001	0.6648	0.0724	<0.0001	0.3102	0.8323	0.8134	0.5367	0.2007
Growth management (GM)	-	-	-	-	-	-	-	0.556	0.0017	0.8508	0.5622	0.2087	0.4097	0.6474
Location	<0.0001	0.0105	0.7903	0.2246	0.5576	0.0075	0.1478	0.9864	<0.0001	0.0165	0.0619	0.9796	0.6647	<0.0001
GM × LM	-	-	-	-	-	-	-	0.7037	0.2181	0.6654	0.8425	0.9809	0.0683	0.1239

	LM biomass							Biological N_2 fixation (Table 5)		SPAD measurements (Figure 3)	
	Before cabbage planting (Table 6)				Before cabbage harvest (Table 7)						
	Dry mass	N concentration	C/N ratio	N content	Dry mass	N concentration	C/N ratio	Before cabbage planting	Before cabbage harvest	Head formation onset	Before cabbage harvest
Living mulches (LM)	0.2385	0.0068	<0.0001	0.1381	0.004	<0.0001	<0.0001	0.1123	0.5782	0.2158	0.3916
Growth management (GM)	-	-	-	-	0.036	0.097	0.032	-	0.039	0.0436	0.4747
Location	0.6823	0.9997	0.5249	0.9246	-	-	-	-	-	0.0001	0.2793
GM × LM	-	-	-	-	0.459	0.013	0.008	-	0.6246	0.4252	0.555

	Cabbage yield (Figures 4 and 5)		N offtake (Figure 6)		
	Head yield	Residues (DM)	Cabbage heads	Cabbage residues	LM biomass (before cabbage harvest)
Living mulches (LM)	0.0003	0.0467	0.0012	0.0402	0.565
Growth management (GM)	0.0800	0.2592	0.1385	0.2546	0.081
Location	0.0079	0.0011	0.0639	0.0061	-
GM × LM	0.0135	0.8095	0.0662	0.4368	0.736

* *p* values correspond to global F tests from the analysis of variance.

Table 4. Soil N_{min} content during the LM and cabbage cultivation period at both locations between March and November (D2–D6) at the depths 0–30, 30–60 and 60–90 cm. Values with at least one identical letter indicate non-significant differences among growth management (capital letters) and LM treatments (lower-case letters) at $\alpha = 0.05$. Values without letters were not found to be significantly different indicated via global F test.

Soil N_{min} kg ha^{-1}	**Start of Vegetation**	**Cabbage Planting**		**Head Formation Onset**		**Cabbage Harvest**	**End of Vegetation**
LM treatments	D2	D3		D4		D5	D6
0–30 cm							
C	9.82	16.6	a	13.5		11.9	15.8
RG	7.63	8.46	c	12.3		12.1	19.2
WC-F	10.1	11.9	b	13.6		14.6	16.7
WC-S	-	18.2	a	15.6		12.1	15.8
MC-F	10.1	11.3	b	14.3		14.9	19.0
MC-S	-	18.7	a	14.6		12.5	15.6
30–60 cm							
C	9.22	14.5	a	9.47	a	5.93	12.7
RG	6.03	7.52	c	5.83	c	5.34	12.5
WC-F	8.33	10.5	b	8.24	ab	5.82	12.3
WC-S	-	15.1	a	9.24	ab	5.87	14.0
MC-F	7.81	10.6	b	7.45	b	5.77	13.9
MC-S	-	14.9	a	8.38	ab	5.74	12.2
60–90 cm							
C	8.83	9.75					5.82
RG	5.04	5.07					5.39
WC-F	8.88	7.17					5.76
WC-S	-	10.9					5.53
MC-F	8.26	7.31					5.88
MC-S	-	10.4					5.80

Table 5. Estimated biological N_2 fixation (BNF) before cabbage planting (both locations) and harvesting (UH). Values with at least one identical letter indicate non-significant differences among growth management treatments at $\alpha = 0.05$. Values without letters were not found to be significantly different indicated via global F test.

Cabbage Planting		**Cabbage Harvest**		
Living mulch [1]	BNF (kg N ha^{-1})	Growth management [2]	BNF (kg N ha^{-1})	
WC-F	51.2	STU	−26.2	A
WC-S	17.1			
MC-F	30.7	STT	−28.1	B
MC-S	26.4			

[1] C = control, RG = perennial ryegrass, WC-F = white clover—fall seeded, WC-S = white clover—spring seeded, MC-F = micro clover—fall seeded, MC-S = micro clover—spring seeded; [2] RT = rototilling, STU = strip till + untreated, STT = strip till + treated.

Table 6. Yield, N concentration, C/N ratio and N content of the living mulch aboveground biomass of both locations shortly before cabbage planting. Values with at least one identical letter indicate non-significant differences among treatments of LM species at $\alpha = 0.05$. Values without letters were not found to be significantly different indicated via global F test.

LM Treatments [1]	**Biomass DM Yield (Mg ha^{-1})**	**N Concentration (% DM)**		**C/N Ratio**		**N Content in Biomass (kg N ha^{-1})**
C	1.87	1.86	bc	19.5	b	31.8
RG	3.46	1.52	c	28.6	a	44.1
WC-F	3.07	2.78	a	14.7	c	78.8
WC-S	0.90	2.76	ac	12.6	c	20.8
MC-F	2.70	2.56	a	15.1	c	62.6
MC-S	0.77	3.11	ab	11.7	c	19.8

[1] C = control, RG = perennial ryegrass, WC-F = white clover—sown in fall, WC-S = white clover—sown in spring, MC-F = micro clover—sown in fall, MC-S = micro clover—sown in spring.

Table 7. Yield, N concentration and C/N ratio of the living mulch aboveground biomass shortly before the cabbage harvest at the location UH. Values with at least one identical letter indicate non-significant differences among growth management treatments (capital letters) or LM treatments (lowercase letters) at α = 0.05.

	Biomass DM Yield ($Mg\ ha^{-1}$)		**N Concentration (% DM)**				**C/N Ratio**			
Growth Management [2]			STU		STT		STU		STT	
LM species [1]										
RG	5.28	a	1.90	b	1.84	c	21.3	a	21.6	a
WC-F	2.16	b	3.47	a	3.27	ab	10.9	b	11.4	bc
WC-S	3.08	b	2.25	b	2.74	b	19.3	a	14.8	b
MC-F	2.03	b	3.49	a	3.41	a	10.6	b	11.1	c
MC-S	2.94	b	2.10	b	2.86	ab	20.3	a	14.1	bc

[1] RT = rototilling, STU = strip till + untreated, STT = strip till + treated; [2] RG = perennial ryegrass, WC-F = white clover—sown in fall, WC-S = white clover—sown in spring, MC-F = micro clover—sown in fall, MC-S = micro clover—sown in spring.

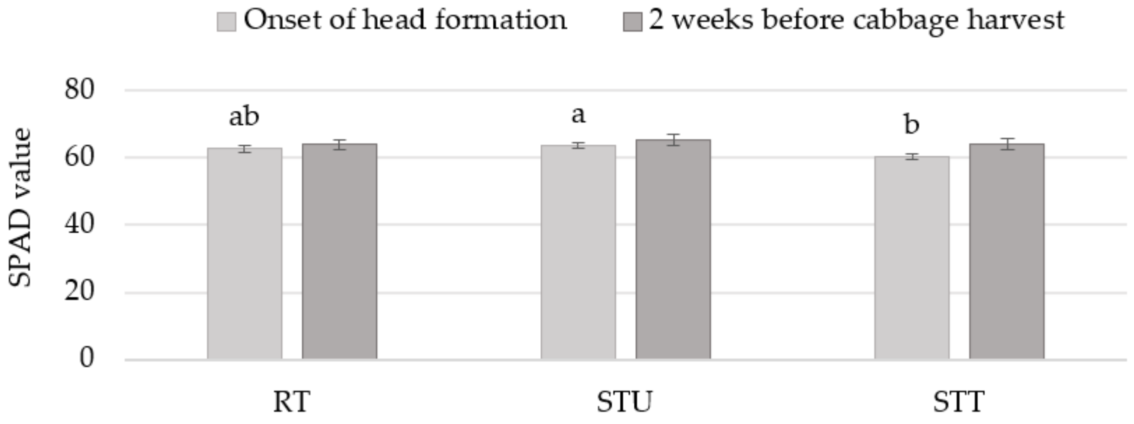

Figure 3. Influence of living mulches and their treatment on SPAD values of oldest, non-senescent leaves of cabbage at the time of head formation onset and two weeks before harvest at both locations. Values with at least one identical letter indicate non-significant differences among LM treatments at α = 0.05. Values without letters were not found to be significantly different indicated via global F test. RT = rototilling, STU = strip till + untreated, STT = strip till + treated.

D1 showed a significant difference between the initial soil N_{min} contents for the two locations (Table 2). For the date D2, none of the differences among the LM treatments were significant (Table 4). Seeing as the treatments WC-S and MC-S were not yet sown at date D2, no soil sampling was performed in these plots. By the time the cabbage was planted (D3), most of the N_{min} values were higher due to higher temperatures and the resulting higher soil mineralization rate. RG had the lowest N_{min} contents, while differences were also evident in the clover treatments sown in the fall. In depths 0–30 and 30–60 cm, both clover treatments sown in the fall showed decreased contents compared to C, WC-S and MC-S.

Further soil sampling took place at the head formation onset (D4). At a depth of 0–30 cm, non-significant differences among the LM could be observed. At a depth of 30–60 cm, among the treatments of the sub-plot factor of LM species, RG sowed the significantly lowest N_{min} content of 5.83 kg ha^{-1} compared to all other treatments. The fall-seeded micro clover MC-F showed lower N_{min} contents than C. The growth management as main effect showed higher N_{min} values when it was rototilled (9.14 kg ha^{-1}) compared to both of the strip till treatments (7.69 and 7.28 kg ha^{-1}, for STU and STT, respectively) at D4 with the depth of 30–60 cm.

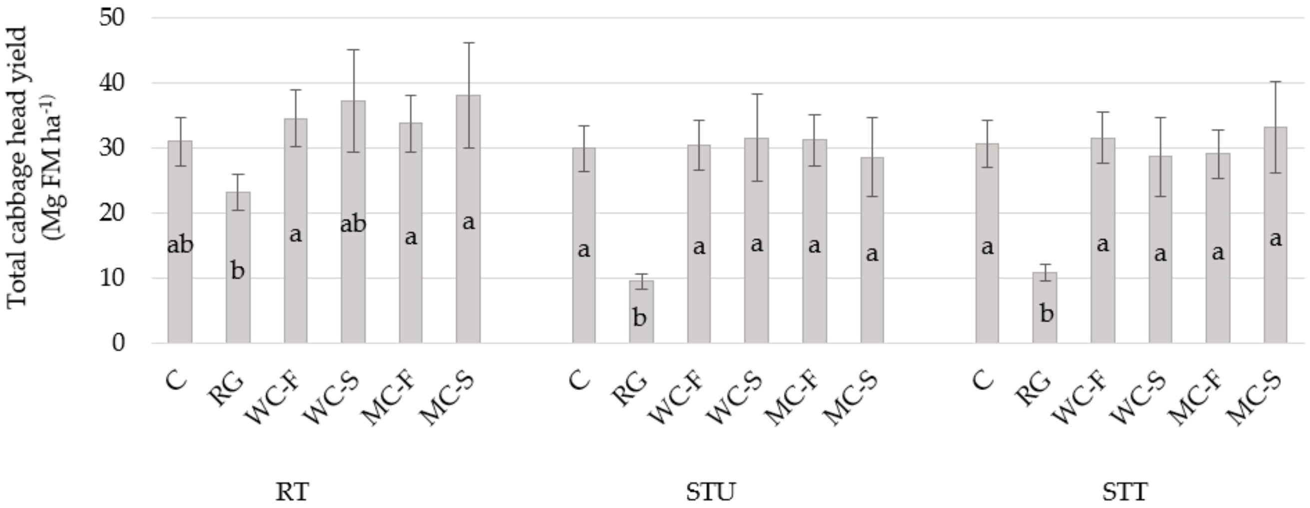

Figure 4. Influence of living mulches on cabbage total head fresh matter yield on both locations. Values with at least one identical letter indicate non-significant differences among LM treatments at α = 0.05. C = control, RG = perennial ryegrass, WC-F = white clover—fall seeded, WC-S = white clover—spring seeded, MC-F = micro clover—fall seeded, MC-S = micro clover—spring seeded; RT = rototilling, STU = strip till + untreated, STT = strip till + treated.

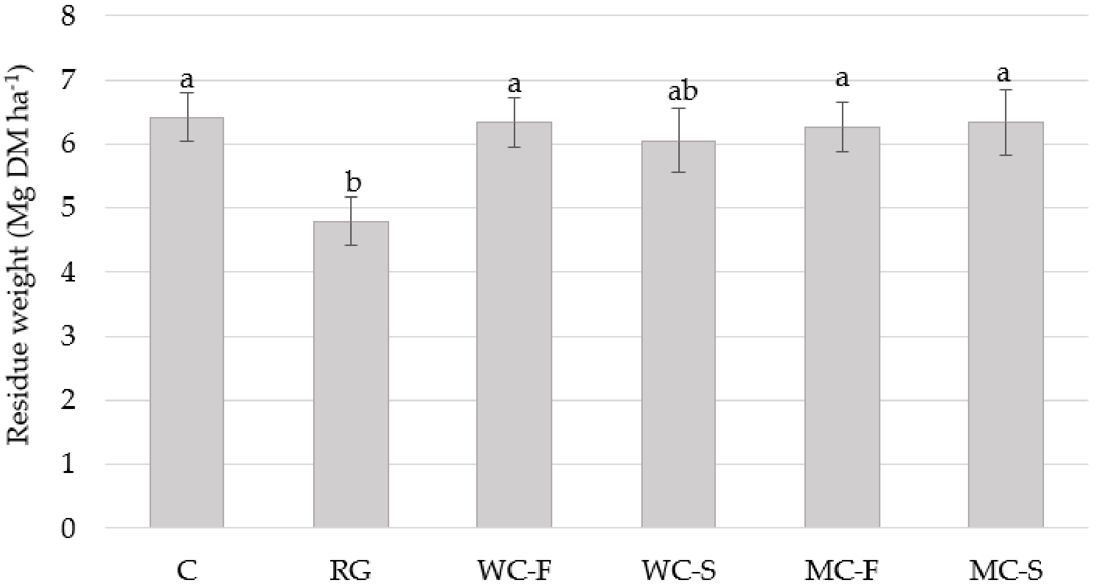

Figure 5. Influence of living mulch treatments on DM biomass of cabbage residues on both locations. Values with at least one identical letter indicate non-significant differences among LM treatments at α = 0.05. RT = rototilling, STU = strip till + untreated, STT = strip till + treated.

At the time of cabbage harvest (D5) and four weeks after the harvest (D6), the difference among the treatments of both factors, growth management and LM species as well as their interaction, were not significant.

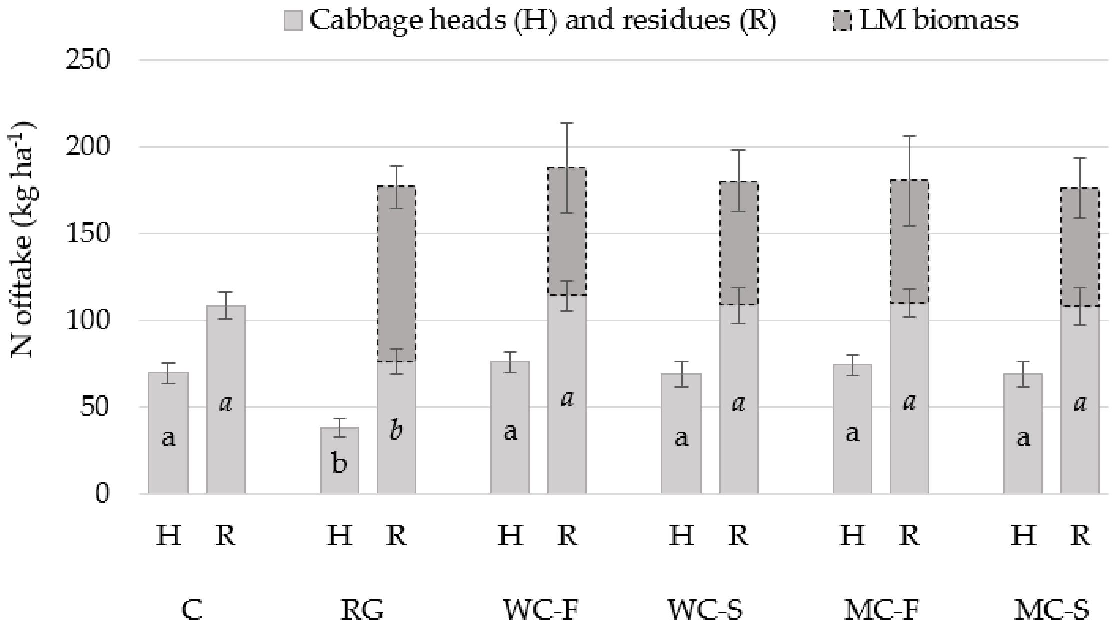

Figure 6. Influence of living mulches on N offtake by harvested cabbage heads (H) and residues remaining on the field (R) with LM biomass (only in case of STU and STT) after harvest at both locations. Values with at least one identical letter (italics for residues) indicate non-significant differences among LM treatments at $\alpha = 0.05$. Values without letters were not found to be significantly different indicated via global F test. C = control, RG = perennial ryegrass, WC-F = white clover—fall seeded, WC-S = white clover—spring seeded, MC-F = micro clover—fall seeded, MC-S = micro clover—spring seeded.

3.2. LM Biomass Growth

Biomass yields and their N contents from the LM treatments were obtained and analyzed shortly before cabbage planting and therefore before the LM were incorporated into the soil of the RT plots. At that time, there was no difference among growth management treatments and therefore, no interaction with LM. However, the LM treatments showed significant differences for N concentration and C/N ratio. The location factor showed no significant differences for the different LM biomass traits (Table 3). RG showed the highest biomass DM yield with 3.83 Mg ha^{-1} followed by the clover varieties sown in fall of the previous year, C with its spontaneous vegetation, WC-S and finally with the lowest biomass DM yield, MC-S (Table 6). Nevertheless, the differences among the LM treatments were not significant. For the N concentration, WC-F and MC-F showed the highest values, which was significantly higher compared to C and RG. Consequently, both C and RG also had significantly higher C/N ratios compared to all four clover treatments, which was even significantly higher for RG than for C. The N content in the biomass was highest in treatment WC-F at 78.8 kg N ha^{-1}. The WC-S and MC-S treatments, that were sown only a few weeks before incorporation, yielded the lowest levels with 20.8 and 19.8 kg N ha^{-1}, respectively. The differences among the LM treatments in terms of N content in biomass were, nonetheless, not significant.

In the second LM biomass assessment at UH for all three years, a significant interaction between growth management and LM in the N concentration trait was detected (Table 7). In addition, there were significant differences in DM among growth management and LM treatments as well as in N concentration among the LM treatments (Table 3). The biomass DM of the STU and STT treatments show that RG yielded significantly more than the clover treatments. Between the growth management treatments, STU had higher yields than STT, 3.58 and 2.62 Mg ha^{-1}, respectively. Regarding the N concentration in the treatment of STU, the clover treatments sown in the fall showed significantly higher values compared to RG and both of the clover treatments sown in spring. Among treatments of STT, all clover treatments showed higher N concentrations than RG, but only MC-F showed higher concentrations than WC-S. The significant differences among LM treatments in N concentration subsequently led to significant differences in C/N ratio. The interaction between growth management and LM species was significant (Table 7), as were the individual factors (Table 3). STT resulted in a significantly lower C/N ratio of 14.2 compared to STU with 15.7. RG had the significantly highest ratio with 21.5; WC-S and MC-S with 16.9 each were significantly higher than WC-F with 11.1 and MC-F with 10.9.

3.3. BNF

In the case of successful clover establishment in the fall (GR 2019, GR 2020, and UH 2021), the differences in the amount of BNF among the LM treatments shortly before the cabbage planting were not significant (Table 5).

Shortly before the cabbage was harvested, the BNF showed negative values in all treatments. No significant differences were found among the LM treatments. Nevertheless, the amount of BNF in the growth management treatment STU was significantly higher as compared to STT (Tables 3 and 7).

3.4. SPAD

For both SPAD measurements during the joint cultivation of LM and cabbage, no significant interaction among the growth management and the LM species could be found (Table 3 and Figure 3). However, the first SPAD measurement taken at the time of head formation onset showed significantly lower SPAD values for the growth management treatment STU compared to RT (Table 3 and Figure 3). At the end of the cabbage cultivation, two weeks before harvest, differences among the treatments of growth management were no longer significant. For both traits, differences among the species were not significant (Figure 3). The location factor showed significant differences for the first but not the second measurement (Table 3).

3.5. Cabbage Yield

The results of the ANOVA regarding the cabbage head yields indicate significant interactions between the factors LM and their growth management, resulting in significant differences among LM depending on the growth management treatment (Figure 4). The location factor also showed significant differences for the cabbage head yield (Table 3). Head yield of all clover treatments within each treatment of growth management was higher than the RG in treatments of STU and STT, while RT and RG had lower head yield only compared to WC-F, MC-F and MC-S. In all treatments of growth management, the clover treatments achieved a head yield comparable to C. In the treatment of RT, the cabbage head yields of the clover varieties tend to be even higher than C. No significant differences in the comparison among C of the different treatments of the main effect growth management in cabbage head yield were found: 31.0, 29.9 and 30.6 Mg ha^{-1} for RT, STU and STT, respectively. The differences of average head yield for the growth management treatments RT, STU and STT were not significant with 32.5 Mg ha^{-1}, 25.8 and 25.0 Mg ha^{-1}, respectively.

The growth management factor and its interaction with the factor LM treatments showed no significant difference in DM biomass for residues. However, the location factor showed significant differences for the cabbage residues' weight (Table 3). Among the LM treatments, RG with 4.79 Mg ha^{-1} had comparatively lower weights than all other LM treatments except for WC-S (Figure 5).

N concentration in cabbage heads and residues (data not shown) showed significant differences among growth management treatments. The concentration was 2.26% for the heads and 1.87% for the residues in STU, which was higher than in RT and STT treatments, with 2.08% and 1.72% as well as 2.07% and 1.73% for the heads and the residues, respectively. However, no other differences, e.g., between locations, could be found.

The N offtake in cabbage heads and residues was significantly lower for RG in all growth management treatments (Figure 6). For the growth management factor, the separated plant parts did not differ in N offtake: There were no significant differences among the treatments RT, STU and STT in cabbage head (72.5, 63.9, and 61.1 kg N ha^{-1}, respectively) or residues (113.4, 98.0, and 101.1 kg N ha^{-1}, respectively). Neither was there an interaction between growth management nor LM species. However, there was a significant difference in the location factor for the cabbage residues (Table 3).

As a result of the combination of lower LM biomass yield and the higher N concentrations of the clover treatments shortly before the cabbage harvest (Table 7), no significant differences in the N content of the biomass could be observed among all treatments.

4. Discussion

4.1. N Supply of Cabbage

The results of this study show that planting cabbage via strip till into LM does not result in a significant reduction in the total head yield. However, there are some signs of competition for light and nutrients by the LM biomass. This is indicated by the lower biomass of cabbage residues compared to previous complete rototilling and incorporation of the LM biomass (Figures 4 and 5). These results are in line with those of Ilnicki and Enache, Hooks et al., Tempesta et al. and Thériault et al. [22,23,25,26]. Some results indicate that the influence of the LM on the N dynamics is the driving force regarding the effects on cabbage growth. For example, in ryegrass treatments, the lowest N_{min} content before planting (Table 4) as well as the lowest N offtakes (Figure 6) were observed. We can exclude effects such as differences in water availability, as water was provided by irrigation. Furthermore, differences in temperature between the two sites are not apparent from Figure 1.

The N supply via horn grit fertilization of 150 kg N ha^{-1} in our work resulted in a cabbage head yield ranging between 9.49 Mg ha^{-1} for RG in STU and 38.1 Mg ha^{-1} for MC-S in RT (Figure 4), and the average head yield was 29.1 Mg ha^{-1}. This is slightly below the yields of Brandsæter et al. [19] (between 27.6 and 53.3 Mg ha^{-1}) and considerably below the yields of Jędrszczyk et al. [20] (between 53.0 and 88.4 Mg ha^{-1}). In both studies, however, the fertilization rates for cabbage planting were 180 and 230 kg N ha^{-1}, respectively, whereas our fertilization rate for cabbage planting was only 150 kg N ha^{-1}. Although Brandsæter et al. [19] call it a suboptimal fertilization rate to avoid masking effects, in their study, the N_{min} content at time of cabbage planting was 83.6 to 125.2 kg ha^{-1} at a depth of 0–40 cm (assuming a DM of the soil of 80% and a density of 1.3 g cm^{-3}). It was, therefore, well above the content of the soil in our study at the time of cabbage planting with 8.46 to 18.7 kg ha^{-1} in 0–30 cm (D4, Table 4). The low N_{min} contents during the vegetation period are the result of very low initial soil N_{min} contents in the fall of the previous year (Table 4). However, low initial soil N_{min} contents are also not unusual for organically managed soils, which is in contrast to the soils of the studies of Brandsæter et al. [19] and Jędrszczyk et al. [20]. Our yields, therefore, line up with the fertilization rate used for cabbage planting in other studies.

However, in our work, there were differences in yield, N offtake of cabbage heads and residue dry matter between the two locations examined (Table 3). These differences were seemingly not caused by fertilization or LM biomass as the fertilization rate was the same and there were no significances in the traits of LM biomass between the locations (Table 3). Furthermore, there were no differences in head and residual N concentrations between the locations (data not shown). With concentrations between 2.07 and 2.26%, these were lower than what Bergmann [38] indicated as sufficient (between 3.7 and 4.5%), but did not qualify as a deficiency. The difference in N offtake of cabbage residues between UH and GR, which is calculated from N concentration and DM yield was, therefore, based on differences in yield. In turn, differences in cabbage yield were more likely to originate from variations in (initial) soil N_{min} content, which would also be supported by the significant location factor (Tables 2 and 3), or from different planting densities (between 31,000 and 42,000 plants ha^{-1}) in UH and GR (Table 2).

Mainly N is the limiting factor in organic vegetable production [39]. However, our results infer that during this study, N supply to cabbage heads was not deficient, showing no significant differences between the intercropping with the previous rototilling of LM. Therefore, neither the N supply of the incorporated LM biomass in the RT treatment nor the longer BNF duration of leguminous LM in the strip till treatments is reflected in the head yield of our results (Figure 4). In fact, the lack of differences in N offtake suggests that all LM treatments, except RG, were able to take up sufficient N and store it in their biomass (Figure 6). One explanation for the lower weight of cabbage residues in STU and STT as compared to RT, but no difference evident in head yield, (Figures 4 and 5) could be the N translocation within the plant. Translocating N from the old parts of the plant (residues) to the young (cabbage head) could compensate for lower N supply to a certain extent. This assumption can be supported by the lower SPAD values at the head formation onset in STU compared to RT, measured on the older cabbage leaves (Figure 3), resulting in lower weights of crop residues. However, there are no differences evident in either the crop residues or in cabbage heads among the growth management treatments of LM in terms of the N offtake (Figure 6).

4.1.1. Green Manure Fertilization via Rototilling

When complete rototillage and strip tillage of LM is compared, it has to be considered that at the time shortly before cabbage planting in the rototilling LM treatment, biomass with a N content of up to 78.8 kg ha^{-1} (Table 6) was incorporated into the soil. Therefore, in the RT treatment, there was an additional fertilization via green manure of the LM. Green manuring due to rototilling and incorporation of the LM biomass further implies that soil tillage, which did not occur in the strip till treatments, might result in an increased mineralization rate in the soil, providing more N to the cabbage plantlets [20]. This difference could probably be compensated by a higher overall N fertilization level than in the present trial. It could be assumed that RT has advantages due to fertilizer effects of leguminous green manures, as indicated by the tendency of higher yields across all leguminous LM in the RT treatment in comparison to the control (Figure 4). This is in line with Thériault et al. [26], who showed that green manuring via rototilling LM (alfalfa and red clover) before planting resulted in higher broccoli yields compared to the LM system. In our study, however, cabbage head yield did not differ significantly depending on LM in the RT treatment. Instead, the higher biomass of residues in RT compared to both treatments of strip stilling, STU and STT (Figure 5) might be related to a higher N supply via green manuring.

Green manure fertilization also occurred in the STU and STT treatments, although at the end of the cabbage cultivation. In addition to the cabbage residues, LM biomass was incorporated after harvest. Here, all treatments with LM showed a N supply of approximately 70 kg more than the control without LM (Figure 6). This additional supply can then be made available to the subsequent crop. The RG treatment could keep up with the clover treatments in this regard, but it contributes more through the high N contents of the RG, which resulted from the high DM yields, than by the high N contents of the crop residues. However, since the C/N ratio of the RG biomass is significantly higher than that of the clover biomass, slower mineralization after incorporation can be assumed here (Table 7).

4.1.2. Biological N_2 Fixation

Another form of N supply occurred through the BNF of legumes. The calculation of BNF for the first LM biomass sampling shortly before cabbage planting (Table 5) suggests that a fall-sown white clover 'Rivendel' (WC-F) contributes more to BNF than a fall-sown micro clover 'Pipolina' (MC-F). The negative values of BNF for the second LM biomass sampling can be explained on the one hand by the high N contents of the reference RG which was derived from the high biomass DM yields rather than high N concentrations in the biomass (Table 7). On the other hand, minor to no differences in soil N_{min} contents between RG and the legumes were found (Table 4). In this context, it must be considered that all treatments were fertilized with 150 kg N ha^{-1} for cabbage planting. This benefits the ryegrass, but may lead to a decrease in BNF; due to the high N supply, the symbiosis is reduced [40]. The average BNF for forage legumes such as clover commonly ranges between 50–250 kg N per ha and year, but due to the short growing period mainly during winter months, these BNF statistics do not match our results (Table 5). Furthermore, the accuracy of the estimation of BNF is rather low with the Stuelpnagel method [35] as it uses the results of plant N and soil N_{min} content of the legumes and the non-leguminous reference perennial ryegrass for the calculation, compared to the recommended and more accurate measurements of symbiotic N_2 fixation using ^{15}N labelled legumes [41].

4.2. Competition between LM and Cabbage

A further research question addressed in this study was whether cutting leguminous LM in the vegetable row will reduce competition with the main crop leading to higher yield compared to untreated LM. An influence on the soil N_{min} content was neither expected nor found by mulching the LM biomass and leaving it between the cabbage rows, e.g., higher N_{min} contents in STU as compared to STT at the time of head formation onset or cabbage harvest (D4 and D5, Table 4). Our findings are therefore in line with the results of Thériault et al. [26] who made the same assumption. However, an indication of reduced competition of LM by mulching could be observed by SPAD measurement at the head formation onset (Figure 3). STT in contrast to STU did not show significantly lower SPAD values compared to RT. Therefore, mulching of LM biomass in the early stage of development appears to reduce intercrop competition. Additionally, a lower biomass DM yield was observed for STT compared to STU shortly before cabbage harvest (Table 3). Thus, it can be assumed that cutting clover biomass can reduce competition between LM and cabbage during the early stages of crop growth. However, it did not supply relevant amounts of N to the growing cabbage crop by mineralization of the biomass. Results from the study of Brandsæter et al. [19] showed that mulching LM biomass does not affect the cash crop yield positively. This is in line with our results as the smaller indications at the beginning and during cabbage vegetation ultimately did not result in a difference between STU and STT in cabbage yield, neither in total head yield nor in crop residues (Figures 4 and 5). On the contrary, the non-mulched STU treatment resulted in significantly higher N concentrations in the cabbage heads and crop residues (data not shown). The reason for this is probably the undisturbed ability of BNF compared to STT, where the fixed N is used to build up the mulched biomass.

As a final research question, we tested whether a dense and low-growing leguminous LM such as micro clover exerts less competition on cabbage plants, compared to a white clover with regular growth, and therefore generates higher cabbage yield. Due to the difficulties in establishing the leguminous LM, only treatments with the same sowing date can be compared. However, no sign of reduced competition from micro clover compared to the white clover with regular growth can be detected in any of the data assessments, on biomass DM of the second cutting date shortly before cabbage harvest (Table 7) or the two SPAD measurements (Figure 3). Our explanation is that there is no actual difference in height or biomass growth between the two varieties.

4.3. Sowing Date and Nitrate Leaching

During the trials, clover treatments were not successfully established in UH in 2019 and 2020 in the fall of the previous year. This could be explained for 2019 by the heavy rainfalls in September (seeding on 6 September 2018) followed by drought, which led to siltation and crusting of the soil. In fact, on 23/09/18, a rainfall of 29.4 L m^2 could be recorded (source www.wetter-bw.de (accessed on 11 March 2022), weather station Hohenheim). In 2020, the clover was assumed to have been sown too deep into the soil.

Due to the difficult establishment of the two leguminous LM, we could assess the influence of the sowing date for the two clover treatments. This primarily affected the N content of the incorporated biomass in the RT treatment (Table 6) and the soil N_{min} content (Table 4) at cabbage planting (D3). The pre-emptive competition of fall-sown LM depleted the soil N_{min} before cabbage planting. The lower N content of spring-sown LM as green manure at the time of LM incorporation in the RT treatment, however, seemed to compensate for the effect of sowing date—at least until the cabbage planting. However, during cabbage cultivation, a significant difference in N concentration and, consequently, C/N ratio between clover varieties sown in the fall and those sown in the spring occurred shortly before cabbage harvest, especially in the STU treatment (Table 7). These results support our assumption that a longer duration of BNF by the clover sown in autumn has an influence on N supply. However, the differences between sowing dates are not reflected in cabbage yield (Figure 4). However, they may be reflected in the N supply or yields of the subsequent crop. Other studies, such as Brainard et al. [13] and Adamczewska-Sowińska et al. [16], only examined sowing dates at or after planting of the vegetable crop and therefore cannot be compared with our results.

However, an early sowing date of the LM also determines the N offtake of LM over winter and thus the potential risk of nitrate leaching. The potential nitrate leaching risk in organic farming is mainly influenced by field management in the fall [12]. Moreover, the amount of N_{min} content in fall has to be considered as the potential for nitrate leaching. It influences the crop growth in the subsequent vegetation period as well as nodule formation on the roots of legumes which determines the BNF [40]. The results of the initial sampling in fall of the previous year (D1) show different N_{min} contents in the soil of the fields for each experimental year conducted (Table 2). In our study, actual leaching was not measured; only the remaining N_{min} content after winter in spring was measured. However, in UH, this leaching potential was very low, especially in fall 2019 and 2020 with N_{min} contents of 38 and 45 kg ha^{-1}, respectively (Table 2). The subsequent samplings D2 and D3 (Table 4) display an expected pattern of N_{min} contents of the individual treatments: RG as a non-legume as well as *Poacea* species shows a strong N offtake and thus reduces the N content in the soil accordingly. To avoid nitrate leaching during winter, RG would be more suitable as a catch crop than clover. As an LM, ryegrass resulted in severe yield losses in cabbage due to poor N supply, visible in LM biomass, N concentration (Table 6), C/N ratio, soil N_{min} contents (Table 4), SPAD values (Figure 3) and cabbage N offtake (Figure 6). In this regard, the low yields in the ryegrass LM treatment in our study are consistent with the results of the works of Müller-Schärer [41] and Adamczewska-Sowińska et al. [16]. Based on these results, we do not consider a LM mixture of clover and ryegrass beneficial given the high yield losses for cabbage despite the ability to reduce nitrate leaching during winter. However, further research on this treatment is recommended for fields with high initial N_{min} values in fall.

5. Conclusions

The successful establishment of a leguminous LM may result in weed suppression, provide additional N by BNF, may reduce nitrate leaching potential during winter and may improve soil properties due to soil cover and organic matter inputs. In the current research work, the impact of water stress on the main crop resulting from LM intercropping could not be evaluated as the cabbage was irrigated. In dry climates, this competition may become more apparent. The LM in vegetable crop stands can be controlled by mulching. However, suitable machinery which can be used for LM growth management in commercial vegetable production systems does not exist yet. The weed-suppressing effect of the LM in contrast to bare soil, depending on the row spacing, is primarily needed until the leaves of the cabbage cover the space between the rows. The use of LM systems may have other advantages that have not been investigated or evaluated in this work, e.g., the actual effects of our LM system on weed suppression, soil protection, or effects of increased biodiversity. N inputs achieved via BNF during the intercropping phase of both crops did not improve

N supply of the main crop, regardless of whether LM was sown in the fall of the previous year or in the spring of the actual year of intercropping with the cabbage. Therefore, the main crop had no direct advantages by the establishment of the LM crop. However, we assume that the longer duration of BNF and the high amount of leguminous biomass of the LM will positively improve the growing conditions of the following crop, e.g., winter wheat. A subsequent cereal crop could additionally reduce the risk of nitrate leaching resulting from increased N input provided by the leguminous LM system.

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