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Impacts of Cover Crops on Early Growth, Nitrogen Uptake and Carbohydrate Composition of Pepper Plants

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Sufficient inorganic nitrogen supply for crop growth is crucial for economically sustainable organic farming. The effects of an application of cover crop biomass on crop growth, nitrogen utilization and carbohydrate composition were investigated during early stage. Short-term changes in soil nitrogen after incorporating fresh hairy vetch and rye shoots were measured. The inorganic nitrogen from cover crops reached the peak at 15 (NH_4-N) and 24 (NO_3-N) days after incorporation, and then decreased rapidly. The highest concentration of soil nitrate showed at 27 days of incorporation in hairy vetch and at 18 days in rye, and three fold differences exhibited between two treatments. Crop growth under hairy vetch or rye incorporation significantly differed. At 20 DAT, dry matter production in NPK and hairy vetch was about two fold greater than that in rye. Difference in decomposing rates of hairy vetch and rye had also influence on nitrogen status in leaves and roots of pepper plants. Total nitrogen was greater in NPK and hairy vetch than in rye until 20 DAT, whereas inorganic nitrogen (nitrate and nitrite) concentration was higher in rye. Temporal changes in soluble sugars and starch in pepper plants among treatments were similar, although difference in the amount existed. It was suggested that hairy vetch as an alternative nitrogen source promoted crop growth and mineral utilization during early growth stage, whereas an obvious effect in rye was not found.

Key words: Cover crop, Growth response, Nitrogen, Carbohydrate

Introduction

Organic or ecological farming systems exclude the use of chemical nitrogen (N) fertilizers, instead requiring a balance between the supply and demand of N through the use of legume-based fertility in the crop rotation (Anonymous, 1991a). Winter grown green manures have been found to reduce nitrogen losses significantly, which secures a higher N supply for succeeding crops (Thorup-Kristensen, 1994; Thorup-Kristensen and Bertelsen, 1996), and they can be assumed to have many other effects on the soil. Legume manure crops, such as hairy vetch, have been known to enrich soil N and increase fruit yield compared with non-legume crop or bare fallow (Abdul-Baki and Teasdale, 1993; Sainju et al., 1999). Non-legume manure crops, such as rye, have been known to increase soil organic C and N (Kuo et al., 1997a). Previous researches with legume- or non-legume cover crop have been mainly performed on soil properties, and crop growth and yield (Korsaeth et al., 2002; Poudel et al., 2001; Sainje et al., 2001). In this research, we studied the effect of legume- or non-legume-cover crop for not only biomass production, but also nitrogen pools and carbohydrate composition in pepper crop during early growth stage.

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Materials and Methods

Field methods and sampling Studies were conducted on an organic farm field at NIAST, RDA. Hairy vetch and rye grass were fall-seeded at a rate of 80 kg and 100 kg ha^{-1}, respectively, in 2005. On May 4, two cover crops were cut and incorporated directly. The biomass of hairy vetch and rye harvested was 31.1 and 30.3 Mg ha^{-1}, both crops included 3.17 and 2.11 % of total nitrogen, respectively. On May 10, pepper 'Manitta' seedlings were transplanted at 27,300 plants ha^{-1} in 90-cm rows. The experimental design was a planned block with three replications. Individual plots were 6 m by 3.7 m and included six rows of pepper. No additional herbicides and fertilizers were added to any of cover crop plots. As comparing with cover crops, NPK plots were fertilized with 158 and 122 kg ha^{-1} of urea and potassium chloride, which were applied with 3- and 2-splits, respectively. As an alternative of P, 200 kg ha^{-1} of poultry manure including 1.22 % of nitrogen was supplied as a basal fertilizer in NPK plots after analyzing soil available P.

Soil sampling and N analysis In order to monitor fluctuations in soil-inorganic- and total-N of pepper-growing field with different N sources, six random soil samples were obtained from 0 to 15 cm soil depth of each plot using auger during 33 days after green manure biomass incorporation. Soil NH_4 (Kopp and McKee, 1978) and NO_3 (Keeney and Nelson, 1982) nitrogen concentrations in the fresh soil were colorimetically determined by flow injection analyzer after extracting with 2 M KCl .

Growth analysis and measurement of nitrogen and carbohydrate The plant was harvested at 10, 20 and 30th day after transplanting, and immediately plant height, dry weight and leaf area were measured. Growth index, relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR), were determined as follows;

$$RGR = 2.303 (\log_{10} W_2 \times \log_{10} W_1) / (t_2 - t_1)$$
$$NAR = \{(W_2 - W_1) X 2.303 (\log_{10} LA_2 - \log_{10} LA_1)\} / \{(t_2 - t_1) (LA_2 - LA_1)\}$$
$$LAR = \{(LA_2 - LA_1) (\log_{10} W_2 - \log_{10} W_1)\} / \{(W_2 - W_1) (\log_{10} LA_2 - \log_{10} LA_1)\}$$

Where:
t = time
W = dry weight
LA = leaf area

Total nitrogen concentration in the plant material was determined by digesting 0.3 g of the samples with 3.3 ml of 368 mM salicylic acid in 84.7 % H_2SO_4 at 300-400℃ for 3-4 h, followed by colorimetry (Walinga et al., 1989). Nitrate and nitrite concentrations were determined as described by Cataldo et al (1975). Briefly, fresh plant sample (0.5 g) was extracted to 5 ml of dH_20 with shaking for 1 h. The resultants were mixed with 2 volumes of 5 % salicylic acid in 97 % of H_2SO_4. Nitrate was measured at 410 nm, calculated according to a standard curve and expressed as μmol NO_3 g^{-1} FW. Nitrite was determined by measuring absorbance at 540 nm after color development for 15 min with a 1 : 1 mixture of 1 % (w/v) sulfanilamide in 1.5 M HCl and 0.02 % (w/v) N-(1-naphtylethylenediamine) dihydrochloride (NED). The NO_2 was expressed as nmol g-1 FW. Soluble sugars were extracted by heating leaf discs in 80 % of ethanol, suggested by Roe method (1955). Soluble sugars were analyzed by the reaction of 1.0 ml of the alcoholic extract with 2.0 ml fresh 0.2% anthrone in sulfuric acid (w/v) and the absorbance was read at 630nm. After the extraction of the soluble fractions, the solid fraction was used for starch analysis. Starch was extracted with 9.3 N perchloric acid first and 4.6 N perchloric acid again. The extracts were combined, and starch concentration was determined after reaction with the anthrone reagent. Glucose was used as standard for soluble sugars and starch.

Data analysis Statistical analysis of data was carried out using ANOVA. The significance of the treatment effect was determined using the Fisher's protected test. To determine the significance of the difference between the means of treatments, least significant difference (LSD) was computed at the 5 % probability level (SAS 8. 12).

Results and Discussion

Average daily temperature during the period measured was lower (18.3℃) when compared with temperature of the last five years (19.3℃, 2000 - 2004) (Fig. 1). Total rainfall during the experiment was greater (67.4 mm) than last five years average (38.5 mm) (Fig. 1). Soil inorganic-N (NO_3-N + NH_4-N) was monitored for 33 days in all treatments (Fig. 2). The release of inorganic-N from cover crops was initiated at 12 days after incorporation (DAI). It reached the peak at 15 (NH_4-N) and 24 (NO_3-N) days after incorporation, and then decreased rapidly (Fig. 2). Nitrate derived from chemical fertilizer increased four folds at 15 days (about 200 mg kg^{-1}) and six folds at 24 days (about 300 mg kg^{-1}) after the application of NPK, and then reduced sharply. Nitrate derived from cover crops was released into soil after 12 days of incorporation, and its concentration was greater in hairy vetch than in rye. For hairy vetch it increased gradually and reached the peak at 24 days (210 mg kg^{-1}),

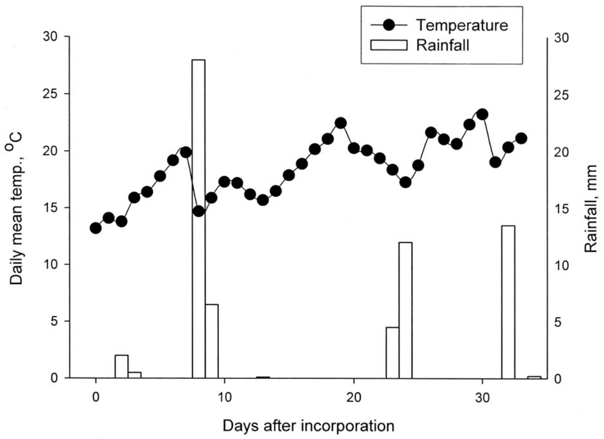

Fig. 1. Average daily temperature and rainfall from May 10 to June 10 in 2005. The scattered line (●) and bar (□) indicate mean temperature and rainfall respectively. Total rainfall during the period examined was 67.4 mm.

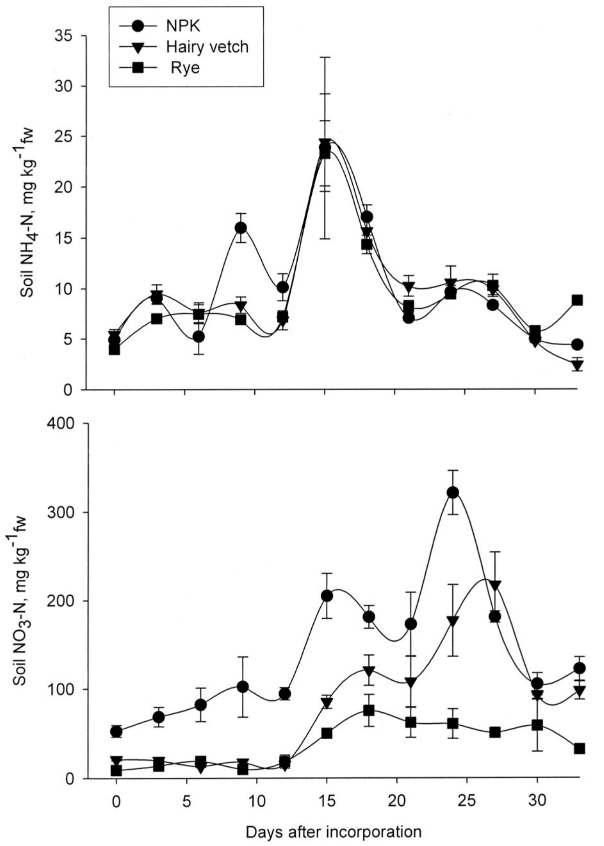

Fig. 2. Changes in soil inorganic N concentration at 0-15 cm depth for 33 days after cover crop incorporation. Error bar represents standard deviation (n = 6).

however, nitrate in rye showed a slight elevation at 18 days (about 60 mg kg^{-1}). Although there are required more detailed examinations, this result can be useful data to decide the time of the side dressing. Net N mineralization during the first week after incorporation was indicated by increases in NO_3^- - N concentration (Lundquist et al., 1999). Nitrogen availability influenced plant biomass production (Table 1). No significant difference was found in biomass production and growth rate among treatments until 10 days after transplanting. A difference in pepper growth among treatments appeared greatly from 10 days. At 20 days after transplanting (DAT), dry matter production and growth rate were not significantly different between NPK- and hairy vetch-pepper system. However rye-pepper system showed half of dry matter production and growth rate when compared with other two systems. The rye-pepper system was superior to NPK- and hairy vetch-pepper systems in RGR and NAR at 30 DAT. Therefore, pepper plants resulted in different growth patterns by applied nitrogen sources, and these results related closely with soil nitrogen status as described previously. The extent of leaf formation and expansion influence the light absorption by the individual leaves within a plant. The enhanced leaf

Table 1. The effects of cover crops on pepper growth at 10, 20 and 30 DAT. The same letter are not significantly different within a column at the 5 % level as determined by Fisher's protected LSD test (n = 3).

Time	Treatment	Dry weight	S:R ratio	RGR	NAR	LAR
		g 10 plants $^{-1}$		$g\ g^{-1}\ d^{-1}$	$g\ m^{-2}\ d^{-1}$	$cm^2\ g^{-1}$
Transplanting	-	4.4±0.1	2.4±0.2	-	-	-
	NPK	8.5±0.6a	3.7±0.2a	0.09±0.01a	3.64±0.38a	236±15a
10 DAT†	Hairy vetch	8.5±0.4a	4.0±0.3a	0.08±0.01a	3.19±0.44a	246±9a
	Rye	8.4±0.6a	2.9±0.1b	0.08±0.01a	3.51±0.29a	221±10
	NPK	32.7±1.4a	6.3±0.2b	0.15±0.01a	8.08±0.42a	183±6a
20 DAT	Hairy vetch	31.5±0.8a	8.0±0.4a	0.15±0.01a	8.03±0.40a	188±1a
	Rye	16.3±1.1b	5.5±0.7b	0.08±0.02b	4.42±0.72b	185±5a
	NPK	122.5±10.4a	9.7±0.4a	0.13±0.12b	5.38±0.69b	222±2a
30 DAT	Hairy vetch	107.6±9.0ab	10.5±1.3a	0.12±0.01b	5.01±0.37b	218±2a
	Rye	88.8±4.1b	11.6±0.6a	0.17±0.01a	7.10±0.50a	234±6a

† Days after transplanting

area has been reported to be the major mechanism leading to compensatory growth. The increase in leaf area is brought about by large N supply by causing the expansion of individual leaves and branches (Gastal and Lemaire, 2002; Trapani and Hall, 1996; Vos and Biemond, 1992). Increase in biomass accumulation is attributed to the increased CO_2 assimilation due to higher rates of photosynthesis by younger leaves (Khan and Lone, 2005). Hairy vetch, which is a low C/N ratio, was better than rye for nitrogen supply and favorable for plant growth at early stage. The nitrogen pools in leaves and roots of pepper plants with different types of N sources were examined (Table 2). Pepper crop responded differently to different nitrogen sources. Inorganic nitrogen in all treatments was greater in shoots than in roots. The pattern of total nitrogen and inorganic nitrogen in pepper leaves was significantly differed at 10 DAT. For leaf nitrogen at 10 DAT, total nitrogen was greater in NPK and hairy vetch than in rye, where inorganic nitrogen was the highest in rye. The concentration pattern of three nitrogen forms was similar in all treatments after 20 DAT. This result was closely related with soil inorganic nitrogen. Nitrogen supply from NPK and hairy vetch favored pepper growth and nitrogen utilization, whereas an insufficient nitrogen supply from rye caused a deficiency of total nitrogen, an excess accumulation of inorganic nitrogen and growth retardation. The content of soluble sugars decreased until 20 DAT in both leaves and roots and was not significantly affected by nitrogen sources (Table 3). Soluble sugars was the most abundant in NPK-applied pepper leaves, while in roots showed highest content in rye-incorporated. Soluble sugars in both tissues increased slightly after 20 DAT. The starch content showed the same pattern with soluble sugars.

Table 2. The effects of cover crops on nitrogen pools in leaves and roots at 10, 20 and 30 DAT. The same letter are not significantly different within a column at the 5 % level as determined by Fisher's protected LSD test (n = 3).

	Leaves			Roots		
	10 DAT†	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
			Total N, %, DW			
NPK	3.50±0.15a	5.12±0.09b	4.68±0.11a	2.01±0.08b	2.85±0.08b	2.71±0.10b
Hairy vetch	3.20±0.03b	5.41±0.11a	4.82±0.10a	2.33±0.03a	3.01±0.10a	3.12±0.07a
Rye	1.78±0.09c	4.84±0.06c	4.86±0.09a	1.52±0.07c	2.20±0.04c	3.06±0.10a
			NO_3^- -N, μmol g^{-1} FW			
NPK	33.1±0.3b	30.7±0.2a	23.8±0.2ab	7.1±0.2b	6.7±0.2c	10.4±0.1a
Hairy vetch	23.4±0.2c	31.3±0.6a	25.4±0.1a	7.1±0.1b	10.4±0.2a	10.3±0.2a
Rye	43.7±0.1a	29.1±1.6a	22.5±1.0b	8.4±0.1a	7.6±0.3b	9.1±0.2b
			NO_2^- -N, nmol g^{-1} FW			
NPK	79±5b	41±3a	16±1b	14±3a	10±1b	11±2a
Hairy vetch	64±4b	37±3a	40±4a	13±3a	20±3a	11±1a
Rye	107±2a	36±3a	30±3a	9±2a	13±1ab	11±3a

† Days after transplanting

Table 3. The effects of cover crops on the synthesis of soluble sugar and starch in leaves and roots at 10, 20 and 30 DAT. The same letter are not significantly different within a column at the 5 % level as determined by Fisher's protected LSD test (n = 3).

	Leaves			Roots		
	10 DAT†	20 DAT	30 DAT	10 DAT	20 DAT	30 DAT
			Soluble sugar, mg kg^{-1} DW			
NPK	11.9±0.5a	7.0±0.2a	9.4±0.2a	2.3±0.1b	1.2±0.1b	2.9±0.1b
Hairy vetch	11.2±0.3a	6.7±0.2a	8.4±0.3b	2.5±0.2b	3.1±0.1a	4.4±0.1a
Rye	11.7±0.4a	6.1±0.5a	8.2±0.1b	3.2±0.1a	1.1±0.1b	2.2±0.1c
			Starch, mg kg^{-1} DW			
NPK	22.0±0.1b	8.4±0.2c	11.6±0.2a	1.5±0.1c	1.2±0.1a	2.1±0.1a
Hairy vetch	27.9±1.7a	15.5±0.1a	14.3±0.4a	2.4±0.1b	0.9±0.1a	2.2±0.1a
Rye	32.7±1.6a	11.1±0.3b	12.8±1.4a	4.8±0.1a	1.1±0.1a	2.1±0.1a

† Days after transplanting

Leaves contained a higher ratio of starch to soluble sugars than roots. This pattern in carbohydrates closely related to plant nitrogen status. Soluble sugars ($r = -0.83$, $p < 0.001$) and starch ($r = -0.92$, $p < 0.001$) in leaves were negatively correlated to total nitrogen. Nitrogen in roots was negative (starch, $r = -0.60$, $p < 0.01$). The availabilities of N (Paul and Driscoll, 1997) influence the photosynthetic rate through the source/sink balance. The accumulation of higher soluble sugars and lower starch with increasing nutrient availability was observed in other species (Koncalova et al., 1993; Cizkova et al., 1996). The content of leaf soluble sugars and starch decreased with increasing N availability in our experiment. The decreasing starch/soluble sugars ratio indicates increasing metabolic activity of the tissue grown under increasing nutrient availability. This situation may signal the competition for carbon between reserve storage and growth (Chapin et al., 1990) and support of N metabolism at the expense of starch accumulation under high N supply (Scheible et al., 1997).

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고추의 초기생장, 질소흡수 및 탄수화물 합성에 대한 녹비작물 시용효과

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유기농업에서 적절한 질소의 공급은 작물생육을 위하여 중요한 일이다. 본 실험은 고추생육초기에 작물생육, 질소이용 및 탄수화물 합성에 대한 녹비작물 시용효과를 알아보고자 수행하였다. 녹비시용 후 30일간 토양 무기태 질소함량을 조사한 결과, 녹비종류에 따라 시용 후 15~25일경에 녹비의 유기태질소는 무기태질소로 대부분 분해되었다. 토양 질산태질소 함량은 헤어리베치 처리구가 호밀 처리구에 비해 3배 가량 높았다. 이러한 질소공급량의 차이는 고추의 생육과 질소흡수량에 큰 영향을 미쳤는데, 정식 후 20일에 측정된 고추 건물생산량은 호밀처리구에 비해 헤어리베치처리구에서 2배 가량 많았으며, 질소 흡수량도 12% 가량 높았다. 생장지수를 고려할 때 호밀 처리구의 고추는 정식 후 20일 이후에 화학비료구 또는 헤어리베치 처리구에 준하는 수준에 도달하였다. 고추 잎과 뿌리의 탄수화물은 정식초기 질소흡수량의 부족으로 체내에 많이 축적되었으나 활착 이후 질소흡수량의 증가는 탄수화물의 이용을 촉진하였다. 따라서 질소비료의 대체원으로 헤어리베치를 기비로서 이용할 경우 정상적인 작물생육을 기대할 수 있으나, 호밀은 느린 분해율로 인하여 작물의 초기생육에 효과적이지 못한 것으로 판단된다.