Original research article

Enhancement of nitrogen uptake in oat by cutting hairy vetch grown as an associated crop

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Abstract: Legume–grass mixed cropping has significant advantages that affect crop yield and soil resources. Generally, grasses grown with legumes take up more nitrogen than those grown under sole cropping. We focused on the effect of cutting hairy vetch during a vigorous growth stage on N uptake in oat under mixed cropping. We evaluated the amounts of N transferred from hairy vetch to oat by using a ^{15}N dilution method. Cutting hairy vetch increased the number of tillers and dry weight of oat, but total N content was not significantly higher than that under mixed cropping without cutting. In contrast, the amount of N transferred to oat was increased by cutting. Estimated amounts of N transferred to oat were 2.7 g m^{-2} with cutting of hairy vetch and 0.8 g m^{-2} without cutting. Cutting half of the oats under sole cropping did not indicate the transfer of rhizodeposited N in oat to the residual plants. In addition, cutting hairy vetch increased the amounts of accumulated solar radiation in the middle canopy of the mixed cropping plots. Therefore, in a hairy vetch and oat mixed cropping system, cutting of the hairy vetch might enhance growth of oat due to the transfer of N from hairy vetch and the reduction of light interception to the oat canopy. N fertility enhancement of the soil by cutting of the legume would be valuable for low-input crop production.

Keywords: cropping system, hairy vetch (*Vicia villosa* Roth), light interception, N_2 fixation, oat (*Avena sativa* L.)

Introduction

Grass–legume mixed cropping is widely used in several agricultural systems, including forage and food crop production. It has significant advantages that affect crop yield and soil resources and has been considered an important practice in low-input crop production aimed at sustainable agriculture (Karpenstein-Machan and Stuelpnagel 2000, Rauber et al. 2001, Ramos et al. 2011). Generally, grasses under mixed cropping with legumes take up more nitrogen (N) than those under sole cropping because of the transfer of rhizodeposited N in the legume to the associated grass. Some pathways of N transfer from the legume to the grass include the death and decay of roots and root nodules of the legumes (Butler et al. 1959, Laidlaw et al. 1996), direct exudation from legume roots (Ta et al. 1986, Paynel et al. 2001), and hyphal links formed by arbuscular mycorrhizal fungi between legume and grass roots (Rogers et al. 2001, He et al. 2009, Li et al. 2009). Of these pathways, N turnover in roots and root nodules could be considered the major source of transferable N in a mixed cropping system of temperate grasses and legumes (Laidlaw et al. 1996). Furthermore, cutting the aboveground parts of the legume during a vigorous growth stage may increase N transferred from the legume to the associated grass through turnover of roots and root nodules (Chestnutt et al. 1980, Daimon and Chujo 1986a, 1986b, Paynel et al. 2001, Paynel and Cliquet 2003), possibly because of the abrupt deprivation of photosynthates in the roots and root nodules (Fownes and Anderson 1991, Chesney and Nygren 2002, Carrillo et al. 2011).

Mixed cropping of winter cover crops, such as hairy vetch or white clover as the legumes and oat or rye as the grasses, has been widely used in non-tillage farming systems. The use of these cover crops as a surface mulch is suggested as an important cultivation technique to maintain soil fertility and prevent soil erosion in crop production (Rosecrance et al. 2000, Gomez et al. 2009, Murungu et al. 2011).

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Under the mixed cropping of hairy vetch and oat, we also showed that cutting the shoots of hairy vetch increased N content in the leaves, number of tillers, and total dry weight of the associated green manure oat crop immediately after cutting (Tarui et al. 2013). However, we could not quantify the amounts of N transferred from the belowground parts of hairy vetch to the associated oat.

The N transferred from the legume to the grass in a mixed cropping system should be quantitatively defined from the viewpoint of an effective use of N fertilizer in a low-input agricultural practice. Several researchers have attempted to quantitatively evaluate N transfer under grass–legume mixed cropping by using a ^{15}N dilution method (Laidlaw et al. 1996, Paynel et al. 2001, Chu et al. 2004, Xiao et al. 2004). Quantitative estimates of the effect of cutting the aboveground parts of legumes on N transfer from the legume to the grass under mixed cropping are limited, although most studies have focused on the contribution of N transfer from the legume to the grass.

In this paper, we focused on the effect of cutting the aboveground parts of hairy vetch on the growth and N uptake in associated oat under mixed cropping. We quantitatively evaluated the amounts of N transferred from hairy vetch to the associated oat by using a ^{15}N dilution method in the field experiment.

Materials and Methods

Experimental site

The experiments were carried out during 2010 and 2011 at the experimental farm of Osaka Prefecture University in Sakai, Osaka, Japan. The soil on the farm is gray lowland soil. Soil pH, electrical conductivity (EC), inorganic N, total N, Truog phosphorus (P), and total carbon (C) were measured before sowing (Table 1). Corn (*Zea mays*) plants had been grown as the previous crop.

Experimental design and measurements

The experiment was laid out in a randomized complete block design with five treatments and three replications. The five treatments were as follows: (1) sole cropping of oat (*Avena sativa* cv. Endax) (A); (2) sole cropping of hairy vetch (*Vicia villosa* Roth cv. Mamekko) (V); (3) mixed cropping of oat and hairy vetch (M); (4) mixed cropping of oat and hairy vetch, which was cut at the early blooming stage (MC); and (5) sole cropping of oat, half of which was cut and used as a control for MC (ACH). Each plot was 1.5 × 2.6 m. The seeds were sown by hand in 6 rows, with an interrow distance of 25 cm and interplant distance of 10 cm, on November 29, 2010. Seeding densities for oat and hairy vetch sole cropping were 92 seeds m^{-2} and 62 seeds m^{-2}, respectively. For the mixed cropping treatments (M and MC), each crop was sown alternately in 6 rows in the plot; hence, mixed cropping plots consisted of 3 rows of hairy vetch and 3 rows of oat, and seeding densities for each crop were half of the sole cropping densities.

The amounts of N derived from the atmosphere and N transferred from hairy vetch were estimated using a ^{15}N dilution method (Chu et al., 2004). Thirty days after sowing, $(^{15}NH_4)_2SO_4$ (10.0 ^{15}N atom%) was applied to a microplot (50 × 75 cm) in the middle of each plot at a rate of 3 g N m^{-2}. Non-labeled ammonium sulfate was applied at the same rate on the margins of the microplots. No other fertilizer was applied.

At approximately 10-day intervals after mid-March 2011, the leaf color of the second expanded leaf below the uppermost leaf was measured by SPAD-502 meter (Konica-Minolta Sensing, Japan) and the number of stems of the oat plants were also measured. The hairy vetch in the mixed cropping plots (MC) and half of the oat plants in the sole cropping plots (ACH) were cut at intervals of one row on April 21, 2011. The cut shoots were removed from the plots to estimate nutrients transferred from the belowground parts of the hairy vetch and half of the oat plants to the associated oat. Two oat plants each from the sole cropping and mixed cropping plots and two hairy vetch plants from the microplots of the mixed cropping plots were sampled for ^{15}N analysis.

After the hairy vetch in the mixed cropping plots and half of the oats in the sole cropping plots were cut, the amounts of accumulated solar radiation in the interrow spaces were measured using sensor film (Optleaf, Taisei Chemical Co. Ltd., Japan) to investigate the aspects of light competition between the two crop species under mixed cropping. The film was set at the center of interrow in each plot at three different heights: uppermost canopy, medium canopy (50 cm

Table 1. Soil chemical properties of the experimental farm

pH (H_2O)	EC (dS m^{-1})	Inorganic N (mg kg^{-1})	Total N (g kg^{-1})	Truog-P (g kg^{-1})	Total C (g kg^{-1})
6.7	0.05	15.3	2.22	1.18	24.9

above the ground), and ground level. Relative amounts of accumulated solar radiation in the interrow spaces were calculated as the values in the medium canopy and at ground level when accumulated solar radiation in the uppermost canopy is 100%.

On May 22, shoots of the oat and hairy vetch grown in all of the plots were harvested. They were weighed and mixed thoroughly, and subsamples of ca. 20% of total fresh weight were analyzed for C, N, and P contents. In addition, two oat plants from the microplots of each plot were sampled for ^{15}N analysis. Hairy vetch shoots sampled from the microplots of the sole cropping plots were also analyzed for ^{15}N contents. They were dried at 70°C for 48 h, weighed, and then ground. Total N contents were measured using an NC analyzer (Vario MAX CN, Elementar, GmbH, Germany), and ^{15}N concentrations were determined using an emission spectrophotometer (YH-5, Shoko Co. Ltd., Japan) according to the method described by Rabie et al. (1980).

Estimation of yields and N transfer under mixed cropping

To estimate the effect of mixed cropping, the advantages of each component crop under mixed cropping and under sole cropping were compared by calculating relative yield (RY) (Caballero et al. 1995, Rauber et al. 2001, Lithourgidis at al. 2006), as follows:

$$\text{RY (oat)} = \frac{\text{Yield of oat under mixed cropping}}{\text{Yield of oat under sole cropping}}$$

$$\text{RY (hairy vetch)} = \frac{\text{Yield of hairy vetch under mixed cropping}}{\text{Yield of hairy vetch under sole cropping}}$$

where Yield is dry weight and N content at harvesting time on May 22. RY >0.5 indicates a positive effect on yields under mixed cropping, and RY <0.5 indicates a disadvantage under mixed cropping.

The percentage and amount of N derived from the atmosphere (Ndfa) in the harvested hairy vetch shoots were calculated using Equations (1) and (2) (Chu et al. 2004):

$$\%\text{Ndfa} = \left(1 - \frac{^{15}\text{N atom\% excess of hairy vetch}}{^{15}\text{N atom\% excess of oat in A}}\right) \times 100 \quad (1)$$

$$\text{Amount of Ndfa} = \%\text{Ndfa} \times \text{total N content of hairy vetch} \quad (2)$$

The calculated N transferred from hairy vetch to oat is expressed as a percentage of N derived from the transferred N from hairy vetch in oat (Ndft). In order to take account of effects of cutting, we used oats in ACH as the reference crop for estimating the N transfer from hairy vetch to oat in MC. The percentage and amount of N derived from transferred N from hairy vetch in oat were calculated using Equations (3) and (4):

$$\%\text{Ndft} = \left(\frac{^{15}\text{N atom\% excess of oat in M (MC)}}{^{15}\text{N atom\% excess of oat in A (ACH)}}\right) \times 100 \quad (3)$$

$$\text{Amount of Ndft} = \%\text{Ndft} \times \text{total N content of oat} \quad (4)$$

Statistical analysis

Data were analyzed using the *t*-test for determining significant differences in the mean of each parameter between treatments, such as mixed cropping and sole cropping, and cutting and the control. A one-way ANOVA and Tukey's test were conducted to determine differences among treatment means of dry weight and total N content of oat sampled before cutting of hairy vetch, when F values were significant.

Results

Growth and N uptake in oat under mixed cropping with hairy vetch

In mid-March, the stem number per plant of oat grown under mixed cropping with hairy vetch (M) was slightly larger than the stem number under sole cropping (A) and increased markedly immediately after cutting hairy vetch at its early blooming stage (MC) (Figs. 1a and 2a). Newly emerged tillers of oat grown under mixed cropping with cutting of hairy vetch (MC) elongated rapidly, and then the dark green leaves unfolded gradually. Although new oat tillers also emerged under mixed cropping (M) and under sole cropping with cutting of half of the oat stands (ACH) in early May, they were few in number (Figs. 1a and 2b). Under sole cropping of oat (A), stem number did not increase and newly emerged tillers did not develop completely (Figs. 1a and 2c). A definite difference was found in the SPAD values of oat leaves between mixed cropping with hairy vetch (M and MC) and sole cropping (A and ACH). The values were higher in both mixed cropping plots than in both sole cropping plots of oat (Fig. 1b).

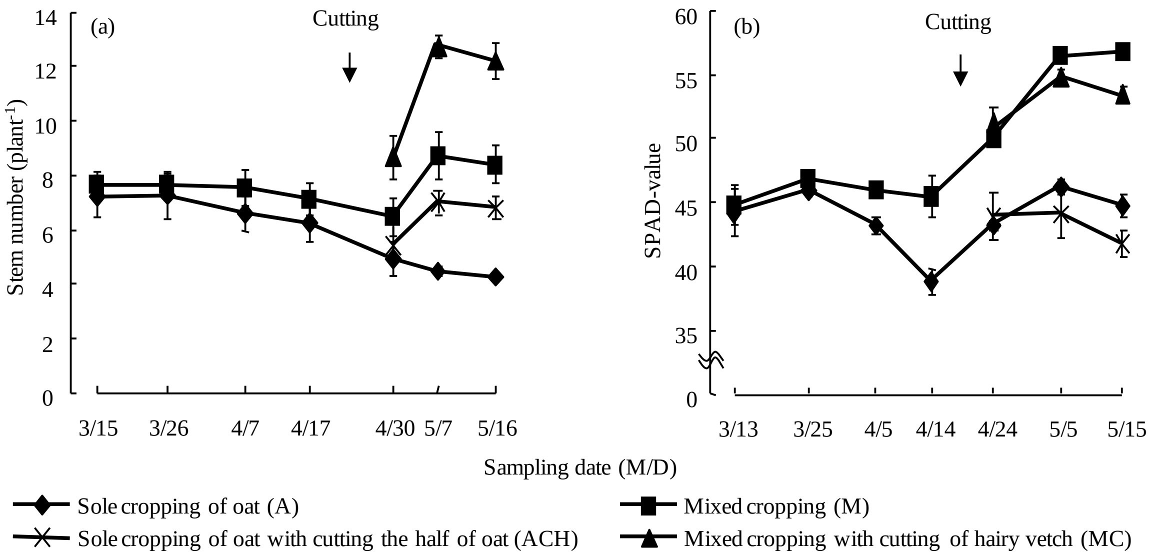

Fig. 1. Changes in stem number (a) and SPAD value (b) of oat plant. Each value shows the mean ± SEM of three replications.

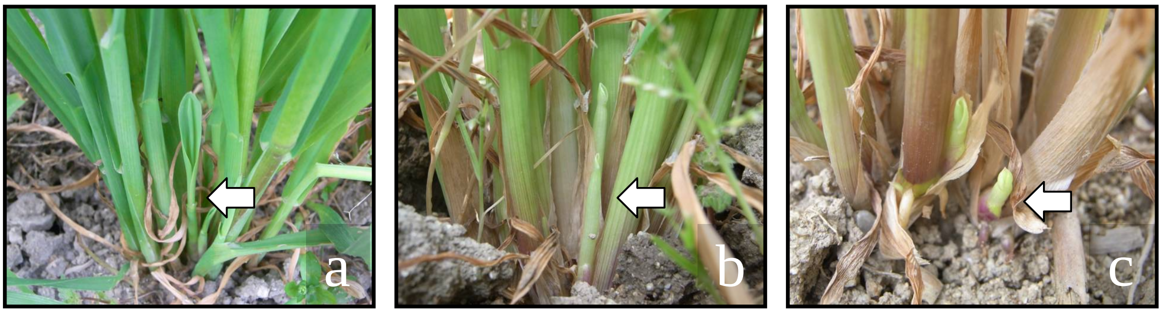

Fig. 2. Differences in growth of newly emerged tillers of oat among treatments on April 30, 2011. Mixed-cropping with cutting of hairy vetch (MC) (a), Sole cropping with cutting the half of oat (ACH) (b), Sole cropping (A) (c)

Cutting the aboveground parts of hairy vetch under mixed cropping (MC) and half of the oat stands under sole cropping (ACH) on April 21 drastically changed the light interception by mutual shading. After cutting the hairy vetch shoots and the oat stands, the relative amounts (%) of accumulated solar radiation in the medium canopy and at ground level were 20–30% higher than those in the plots without cutting (Fig. 3). In the mixed cropping plots (M), values in the medium canopy decreased gradually after late April and remained markedly lower (13–23%) at ground level. In these plots, oat plants competed with hairy vetch vines for light (Fig. 4a and Fig. 5).

At the early blooming growth stage of hairy vetch on April 21, there was no significant difference in the shoot dry weight of oat between sole cropping (A) and mixed cropping (M) (Table 2). However, the total N concentration of oat shoots under mixed cropping (M) was significantly higher than that under sole cropping (A). The dry weight and total N content of the removed hairy vetch shoots were 113.7 g m^{-2} and 3.7 g m^{-2}, respectively.

The values of RY (oat) in dry weight and total N content of oat shoots under mixed cropping (M) at harvesting time were 0.58 and 0.79, respectively. The values under mixed cropping with cutting of hairy vetch (MC) were 0.69 and 0.83, respectively, whereas those under sole cropping with cutting of half of the oat stands (ACH) were 0.46 and 0.50, respectively (Table 3).

There were also differences in growth and N uptake in hairy vetch shoots between sole cropping and mixed cropping with oat. The values of RY (hairy vetch) in dry weight and total N content of hairy vetch shoots under mixed cropping were 0.70 and 0.73, respectively (Table 4).

Amounts of N fixed by hairy vetch and transferred to oat under mixed cropping

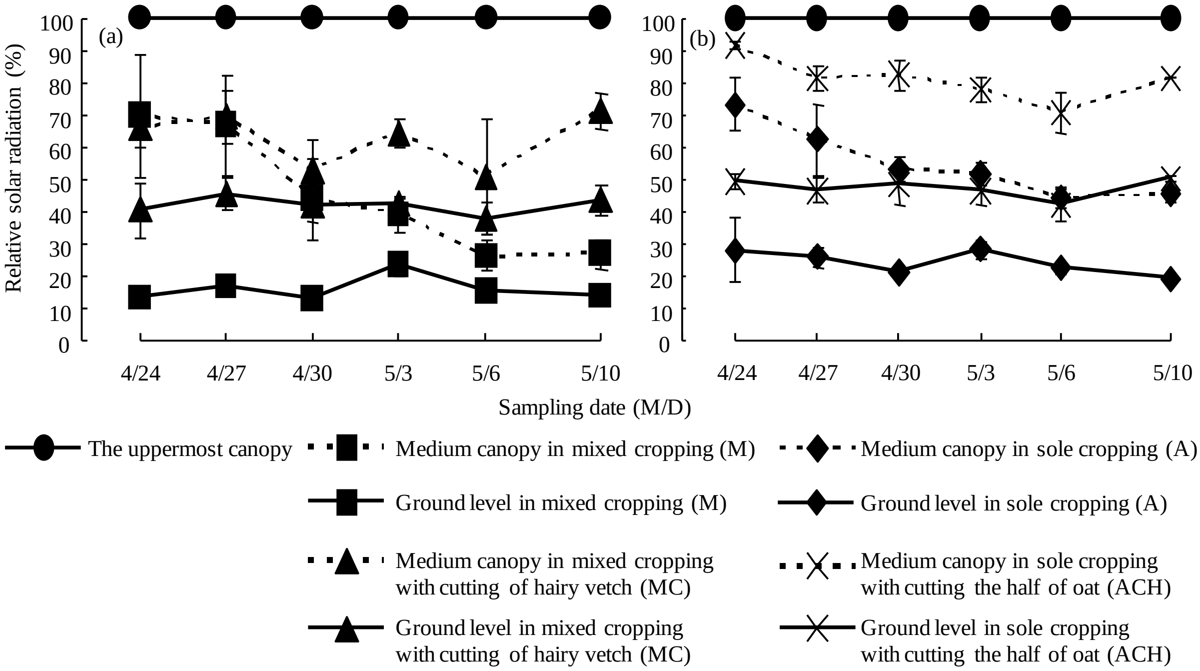

Fig. 3. Effect of cutting hairy vetch in mixed cropping (a) or the half of oat in sole cropping (b) on relative amount of accumulated solar radiation at the interrow of crop stands. Each value shows the mean ± SEM of three replications.

Fig. 4. Covering the interrow space by hairy vetch in mixed-cropping with oat plants on April 28, 2011. Hairy vetch vines twined around oat (M) (a) , Reduction of the light interception after cutting of hairy vetch in mixed-cropping (MC) (b)

In the present experiment, quantification of the amounts of N fixed by hairy vetch and N transferred from hairy vetch to oat under mixed cropping was conducted by using a ^{15}N dilution method (Chu et al. 2004). Before cutting the aboveground parts of hairy vetch at the early blooming stage, small amounts of N were transferred to the associated oat under mixed cropping (M) (0.6 g m^{-2}) (Table 5). The percentage of N derived from the atmosphere in the total N amounts in hairy vetch at later blooming stages was 93.5% under sole cropping (Table 6), indicating that hairy vetch had a higher potential of N fixation in the present field experiment.

After cutting the aboveground parts of hairy vetch, ^{15}N atom% excess of oat in mixed cropping with hairy vetch cutting (MC) (0.973 atom% excess) significantly was lower than that in mixed cropping without cutting (M) (1.393 atom% excess), resulting that larger amounts of N were transferred to the associated oat (MC) (2.7 g m^{-2}) compared with mixed cropping without cutting (M) (0.8 g m^{-2}) (Table 6). The percentage of transferred N in the total N amounts in the associated oat (Ndft) was also significantly higher under mixed cropping with cutting of hairy vetch

Fig. 5. Climbing of hairy vetch vines around the oat plants in mixed cropping (M) on March 28, 2011

(MC) (38.0%) compared with mixed cropping without cutting (M) (11.6%) (Table 6). In contrast, cutting half of the oat plants in the sole cropping plots (ACH) did not show a change in ^{15}N atom% excess in the residual oat.

Discussion

In our previous study on mixed cropping of hairy vetch and oat, cutting the aboveground parts of hairy vetch during the vegetative growth stage considerably increased dry matter production and N absorption in the associated oat (Tarui et al. 2013). In the present experiment, we confirmed the enhancement of N uptake in the oat crop under mixed cropping with hairy vetch. This might be explained by N transfer from the associated hairy vetch. Root nodules of the legume often decayed immediately after cutting the aboveground parts because of the deprivation of photosynthates to the roots. Fownes and Anderson (1991) reported that living root nodule biomass decreased immediately after cutting shoots of *Sesbania sesban* and *Leucaena leucocephala*, and then dead nodule biomass increased. Cutting the aboveground parts of neighboring oat stands as a control (ACH) in the present experiment did not promote N uptake after cutting (Fig. 1b and Table 3). We assumed that N derived mainly from hairy vetch root nodules could be supplied to the associated oat, because hairy vetch accumulated fixed N in the root nodules during the vegetative growth stage.

In grass-legume mixed cropping, N absorbed by the neighboring grass in mixed cropping with legume is generally derived from fertilizer, soil, and the associated legume (fixed N). When N fertilizer is not applied to the field, fate of N between the grass and the legume could be analyzed for the two derivations, soil N and fixed N. As no fertilization plot was not made in the present experiment, we could not analyze the difference in N absorbed between these two derivations. On the other hand, utilization of ^{15}N labeled fertilizer enabled the quantification of N fixed by legume. Fate of N through cutting hairy vetch in mixed cropping with oat should be further analyzed under various conditions of soil fertility.

Thorsted et al. (2006) indicated that vigorous growth of the associated grass under mixed cropping was due to not only N transfer from the legume but also the reduction of light interception by cutting the legume. The light interception of the canopy reduced tiller initiation in switchgrass (Williamson et al. 2012) and induced tiller cessation in the wheat plant (Evers et al., 2006). In the present experiment, cutting the aboveground parts of hairy vetch markedly increased the amounts of accumulated solar radiation in the middle canopy and at ground level, and it might induce increases in stem number in the oat plants grown under mixed cropping (Figs. 1a and 3a). In addition, cutting the aboveground parts of oat as a control also increased the amounts of accumulated solar radiation in the middle canopy and at ground level, and it induced a slight increase in stem number of the oat plants (Figs. 1a and 3b). Therefore, under mixed cropping of hairy vetch and oat with cutting, both N transfer from the hairy vetch and the reduction of light interception to the oat canopy might enhance growth of the associated oat. Especially, changes in relation between solar radiation and dry matter accumulation of the associated oat should be further analyzed for understanding influence of cutting.

Table 2. Dry weight and total N content of oat shoots sampled at April 21, 2011

Treatment	Dry weight	Total N content	
	(g plant^{-1})	(mg g^{-1})	(mg plant^{-1})
Sole cropping (A)	17.1	10.4	177.8
Mixed cropping (M)	15.3	15.4	235.6
t-test	ns	*	ns

* : Significant difference at 5% level
ns : not significant difference

Table 3. Effect of cutting hairy vetch at blooming stage on dry weight and total N content of oat shoots sampled at May 22, 2011

Treatment	Dry weight		Total N content		
	($g\ m^{-2}$)	RY	($mg\ g^{-1}$)	($g\ m^{-2}$)	RY
Sole cropping (A)	970a	-	8.9b	8.6a	-
Sole cropping of oat with cutting the half of oat (ACH)	444d	0.46c	9.6b	4.3c	0.50b
Mixed cropping (M)	560c	0.58b	12.2a	6.8b	0.79a
Mixed cropping with cutting of hairy vetch (MC)	667b	0.69a	10.7ab	7.1ab	0.83a

Values in the same column followed by same letter are not significantly different at 5% level according to Tukey’s test.
RY: relative yield
- : no calculation can be done.

Table 4. Effect of mixed cropping with oat on dry weight and total N content of hairy vetch sampled at May 22, 2011

Treatment	Dry weight		Total N content		
	($g\ m^{-2}$)	RY	($mg\ g^{-1}$)	($g\ m^{-2}$)	RY
Sole cropping (V)	574	-	28.6	16.4	-
Mixed cropping (M)	403	0.70	29.7	12.0	0.73
t-test	ns	-	ns	ns	-

ns : not significant difference.
RY: relative yield
- : no calculation can be done.

Table 5. Percentage of N derived from atmosphere (Ndfa) in hairy vetch and N derived from transferred N from hairy vetch in oat (Ndft) sampled at April 21, 2011

Plant	^{15}N atom% excess	Ndfa		Ndft	
		(%)	($g\ m^{-2}$)	(%)	($g\ m^{-2}$)
Oat in sole cropping (A)	1.762	-	-	-	-
Oat in mixed cropping (M)	1.491	-	-	15.4	0.6
t-test	ns	-	-	-	-
Hairy vetch in mixed cropping (M)	0.240	86.4	7.3	-	-

ns : not significant difference
- : no calculation can be done.

On the other hand, the climbing of hairy vetch vines on the oat plants might induce positive effects on biomass production of this crop, resulting in higher RY values in hairy vetch under mixed cropping (M) compared to sole cropping (V) (Fig 5, Table 4). As Rauber et al. (2001) reported that pea lodging was prevented in mixed cropping with oat because oat plant acted as a prop for pea vines, climbing legumes often twine around the associated crop in mixed cropping.

There was a significant difference in ^{15}N atom% of oat plants harvested on May 22 among sole cropping, mixed cropping with hairy vetch, and mixed cropping with hairy vetch and cutting of the shoots (Table 6). According to our estimation using a ^{15}N tracer technique in this experiment, the amounts of transferred N in the total N content of the associated oat (Ndft) under mixed cropping (M) and under mixed

Table 6. Percentage of N derived from atmosphere (Ndfa) in hairy vetch and N derived from transferred N from hairy vetch in oat (Ndft) sampled at May 22, 2011

Plant	^{15}N atom% excess	Ndfa		Ndft	
		(%)	(g m^{-2})	(%)	(g m^{-2})
Oat in sole cropping (A)	1.575	-	-	-	-
Oat in sole cropping of oat with cutting the half of oat (ACH)	1.569	-	-	-	-
t-test	ns	-	-	-	-
Oat in mixed cropping (M)	1.393	-	-	11.6	0.8
Oat in mixed cropping with cutting of hairy vetch (MC)	0.973	-	-	38.0	2.7
t-test	*	-	-	*	*
Hairy vetch in sole cropping (V)	0.103	93.5	15.3	-	-

* : Significant difference at 5% level.
ns : not significant difference
- : no calculation can be done.

cropping with cutting of hairy vetch (MC) were 0.8 and 2.7 g m^{-2}, respectively. The percentage of transferred N in the total N content of oat (%Ndft) under mixed cropping was significantly lower (11.6%) than that under mixed cropping with cutting of hairy vetch (38.0%). Sierra and Desfontaines (2009) reported that cutting the shoot of the jack bean increased the percentage of transferred N in the total N content under mixed cropping with banana as the roots of the jack bean decomposed. They estimated 32% N transfer, and our results coincided with their result.

Cutting the aboveground parts of legumes under mixed cropping with grass might provide a novel practice for fertilization management in those cultivation systems. Thorsted et al. (2006) reported that the N concentration in wheat grain increased after cutting the aboveground parts of mixed cropping white clover at the flag leaf stage of the wheat. Considering that top dressing of N fertilizer improves the bread-making quality of wheat in Japan, N fertility enhancement of the soil by the aboveground cutting of legumes grown under mixed cropping would be valuable for organic wheat production, where chemical fertilizers cannot be used as top dressing. Mixed cropping using hairy vetch may be an effective cultivation practice for organic grain production. Several problems remain before this cultivation system can be applied to an actual technique for growers. For example, further experiments on the mechanized cutting of legume shoots grown under mixed cropping with grass should be conducted using several seeding densities. In addition, more efficient and less costly cutting management practices must be developed.

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