

Dynamics of N Derived from ¹⁵N-labeled Rye in Soil–tomato System as Influenced by Cover Crop Residue Management

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The nitrogen (N) contribution of rye (Secale cereale L.) to tomato production may increase when grown and applied with hairy vetch (Vicia villosa R.) to the soil. To examine the uptake and recovery efficiency by tomatoes and retention in the soil of N derived from ¹⁵N-labeled rye applied as a monoculture and biculture with hairy vetch, a Wagner pot examination was conducted under plastic high tunnel conditions in Sapporo, Japan. Irrespective of cover crop residue management, the peak of rye-derived N uptake occurred between 4 and 8 weeks after transplanting (WAT) and ceased between 8 and 12 WAT. Rve-derived N uptake by tomatoes (shoot + fruit) was 58.3% greater in rye monoculture treatment than in the biculture of hairy vetch and rye treatment because of higher rye-derived N input, whereas rye-derived N recovery was greater in the biculture treatment (34.0%) than in monoculture treatment (26.9%). The soil retained 47.0% and 52.5% of the ryederived N input in the biculture (972 mg N/pot) and rye monoculture (1943 mg N/pot) treatments, respectively. Rye-derived N stored in the roots and possibly lost was estimated at 19.0% and 20.6% of the rye-derived N input in the biculture and monoculture treatments, respectively. Hairy vetch in the biculture treatment contributed 46.2% more N to tomato production than rye, and the hairy vetch N contribution was more significant during the late period (4–8 WAT) than the early period (0–4 WAT) of tomato cultivation. Therefore, the biculture may change the N release pattern from both hairy vetch and rye, with the cover crops releasing high amounts of N in both the early and late periods of tomato cultivation. These results may help improve N management in vegetable production systems by maximizing the use of plant-derived N by crops, thereby reducing N fertilizer inputs.

Key Words: biculture, hairy vetch, N recovery, N uptake, soil N retention.

Introduction

Nonlegume cover crops such as cereal rye (*Secale cereale* L.) do not add N to the cropping systems but help conserve N through crop uptake and immobilization (Clark et al., 1994; Meisinger et al., 1991; Shipley

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et al., 1992). Because of faster and extensive root growth, rye may scavenge residual nitrate, subject to leaching losses, better than legumes (Sainju et al., 1998). As opposed to rye, hairy vetch (Vicia villosa R.) can fix atmospheric N and add large amounts of N to the cropping systems, resulting in high soil N availability and crop yield compared with rye or bare fallow (Clark et al., 1994; Kuo et al., 2001). However, because of a high N concentration and low C/N ratio, hairy vetch decomposes rapidly after incorporation in the soil, releasing large amounts of N in the first 4 weeks after incorporation of residues in the soil (Sugihara et al., 2013). Too early N mineralization may result in low tomato N recovery due to lack of synchrony between N release and tomato N demand (Hartz and Bottoms, 2009). Moreover, the likelihood of N loss (leaching and/or denitrification) is greater with plant

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residues releasing N rapidly than slowly (Muller and Sundman, 1988).

Alternatively, the biculture of hairy vetch and rye, with an intermediate C/N ratio between hairy vetch and rye, may show a moderate decomposition rate that may result in high tomato N recovery. In our earlier study, tomatoes growing in the biculture of hairy vetch and rye showed greater residue-derived N recovery and tomato fruit yield than those growing in hairy vetch monoculture (incorporation). In contrast, tomatoes in rye monoculture showed the lowest growth and tomato yield among cover crop treatments because of N immobilization (Muchanga et al., 2020). These findings suggest that rye contribution to soil inorganic N and tomato yield may improve when rye is grown and incorporated with hairy vetch in the soil. Therefore, rye may play an important role in N management in cropping systems and help increase residue-derived N recovery by crops, thereby increasing crop yields while reducing N fertilization rates. However, studies about rye-derived N dynamics in soil-plant systems are very limited, possibly because of the detrimental effect of rye on crop yields (Clark et al., 1994; Sainju et al., 2003). Therefore, little is known about rye-derived N dynamics in soil-tomato systems. On the other hand, dynamics of N derived from ¹⁵N-labeled legumes, especially hairy vetch, in crop production (including tomatoes) have been wellstudied (Cueto-Wong et al., 2001a; Hadas et al., 2002; Seo et al., 2006; Sugihara et al., 2013).

The objective of this study was to understand the reasons for the high effectiveness of the biculture of hairy vetch and rye in increasing fresh-market tomato yield observed in our previous study (Muchanga et al., 2020) by examining the uptake and recovery efficiency by tomatoes and retention in the soil of N derived from rye residues applied to the soil as a monoculture and biculture with hairy vetch. Because the C/N ratio of the biculture of hairy vetch and rye is lower than that of rye only, the research hypotheses were that: (1) the uptake and recovery of rye-derived N by tomatoes would be greater with the biculture than the rye monoculture, and (2) rye-derived N retention in the soil would be greater with rye monoculture than the biculture.

Materials and Methods

The site and experimental design

The experiment was conducted in a plastic high tunnel at Experimental Farm of the Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, in 2018. The chemical properties of the Gleysol soil (clay loam texture) used in pots were as follows: 65.2 mg NO₃⁻-N, 18.9 mg NH₄⁺-N, 0.78 g K, 3.14 g Ca, 0.46 g Mg, and 0.40 g P (Truog-L method), per kg dry soil. The total C and N were 4.64% and 0.36%, respectively; and the soil electrical conductivity (EC) and pH were $61.9 \text{ mS} \cdot \text{m}^{-1}$ and 6.3, respectively. The treatments consisted of rye cover crop (RYE), hairy vetch cover crop (HV), a mixture of hairy vetch and rye cover crops (HV+RYE), and a no-cover crop treatment (BARE). The levels of total organic N applied in HV, RYE, and HV+RYE were similar: 1943 mg N was applied in each $1/2000 a (0.05 m^2)$ Wagner pot; and the ratio of hairy vetch-derived N to rye-derived N in HV +RYE was 1:1 (Table 1). No supplemental N fertilizer was applied to any treatment. The treatments were arranged in a randomized complete block design with each treatment repeated 5 times (5 pots). Each treatment consisted of 20 pots, with 5 pots collected on each sampling date. The mean soil and air temperatures in the pots measured with a data logger (Logger GL820; GRAPFTEC Corporation, Yokohama, Japan) during the period of tomato cultivation were 21.5°C and 22.2°C, respectively.

¹⁵N-labeled rye and hairy vetch cultivation, tomato transplanting, and management

Rye 'Fuyumidori' seedlings (HOKUREN Federation of Agricultural Cooperatives, Hokkaido, Japan) planted on 24 Sept. 2017 in the open-field were collected and transplanted in Wagner pots filled with soil on 20 Apr. 2018 in a plastic high tunnel. After transplanting, water was applied by an irrigation tube as needed until the beginning of the heading stage. Two weeks after transplanting, 10 to 15 mL of $0.5 \text{ g} \cdot \text{L}^{-1}$ ¹⁵NH₄Cl (98% ¹⁵N atom% excess) (Sugihara et al., 2013) was applied to rye pots every week a total of five times. Hairy vetch 'Mamesuke' (Snow Brand Seed Co., Ltd., Sapporo, Japan) was sown in a field plot (3 m²) in a plastic high tunnel and irrigated as needed until the flowering stage.

Table 1. Nitrogen concentration, C/N ratio, isotope ratio, and total N from hairy vetch and rye applied to the pots.

		Hairy vetch			Total Namplied				
Treatments	DW (g)	N concentration (%)	N applied (mg N/pot)	DW (g)	N concentration (%)	N applied (mg N/pot)	¹⁵ N atom% excess ^z	(mg N/pot)	C/N ratio
HV	44.3	4.39	1943				_	1943	9.69
RYE	_	_		81.4	2.39	1943	0.27	1943	17.4
HV+RYE	22.1	4.39	972	40.7	2.39	972	0.27	1943	13.6

^z Analyzed from the aboveground biomass.

No fertilizer was applied to hairy vetch. Rye grown in pots was mowed about one week after the beginning of the heading stage and hairy vetch was mowed at the beginning of the flowering stage (10% of plants heading or flowering). After mowing of cover crops, above-ground biomass was collected and chopped into small pieces that were incorporated in Wagner pots filled with 4.3 kg dry soil on 12 June. P and K fertilizers were applied in conjunction with cover crop residues as fused calcium magnesium phosphate (29%Ca-7%Mg-7%P) and potassium sulfate (42%K-17%S) at a rate of 200 kg·ha⁻¹ each, respectively.

Tomato 'Reika' (SAKATA SEED CORPORATION, Yokohama, Japan) was sown in plastic boxes (43×33) \times 7 cm) on 3 Apr. 2018; two weeks after sowing, seedlings were transferred into plastic pots of 12 cm in diameter, where they grew until the emergence of the first flower cluster. Two and half-month-old tomato seedlings were transplanted into Wagner pots on 15 June. After transplanting, water was supplied to tomatoes using a plastic cup. Tension meters (DIK-8330; Daiki Rika Kogyo Co., Ltd., Kounosu, Japan) were randomly set in some pots to monitor soil moisture. Tension meters were monitored daily and irrigation (1 to 1.5 L/pot was applied to avoid N leaching) was carried out when the pF value was higher than 2.5 (Araki and Fujii, 2013). For trellising tomato plants, a hangingstring system was established just after transplanting. Weeds were removed by hand, shredded, and returned to the same pots. Pesticides were applied twice to control fruit worms.

Sample collection and analyses

A week prior to cover crop mowing, a portion of hairy vetch and rye residues was collected, oven-dried at 60°C, and ground with a sample mill (T1-100; CMT Co., Ltd., Iwaki, Japan) to determine C and N concentrations by an elemental analyzer (Vario EL III; Elementar, Hanau, Germany). The N concentrations of rye and hairy vetch were 2.39% and 4.39%, respectively (Table 1). Five tomato plants per treatment were collected at 2, 4, 8, and 12 weeks after transplanting (WAT) and their fresh weight (FW) was taken soon after. After collection, tomato plants were divided into shoot and fruit, which were both oven-dried at 60°C to determine their dry matter content that was used as a conversion factor to calculate the shoot biomass or fruit yield. Fruit that reached the maturity stage (red-ripe) before the sampling date was harvested immediately to avoid loss, and treated as described earlier. In order to monitor soil inorganic N levels $(NO_3^{-}-N + NH_4^{+}-N)$ following the incorporation of cover crop residues, soil samples were taken at 1, 2, 4, 8, and 12 WAT. NO₃-N and NH_4^+ -N were measured by a soil analyzer (ZA-II; Fujihira Industry Co., Ltd., Tokyo, Japan) following alkali reduction-diazotization and indophenol methods, respectively. The soil samples collected at 2, 4, 8, and

12 WAT were also analyzed for soil total N (STN) by an elemental analyzer (Vario EL III; Elementar, Hanau, Germany). Soil samples were collected from five pots in each treatment and then composited. Composited samples were air-dried and sieved with 2-mm mesh before analyses. Tomato shoot and fruit, rye (above-ground), and soil samples were also analyzed for ¹⁵N abundance by an elemental analyzer (Flash Flash2000–DELTA^{plus} Advantage ConFlo III System; Thermo Fisher Scientific Inc., Waltham, MA, USA). The ¹⁵N atom% excess of rye residues was 0.27% (Table 1).

Calculations

The proportion of total N in tomato or in the soil derived from rye residues (%RdN) (1), rye-derived N recovery efficiency (2), and rye-derived N in the soil (soil–RdN) (3) were determined as follows:

- (1) %RdN = ¹⁵N atom% excess in tomato or soil/¹⁵N atom% excess in rye cover crop × 100
- (2) Rye-derived N recovery efficiency (%) = ryederived N uptake/rye-derived N input × 100
- (3) Soil-RdN (mg $N \cdot kg^{-1}$) = STN (mg $N \cdot kg^{-1}$) × %RdN/100

Rye-derived N uptake was determined as the positive difference of the shoot or fruit rye-derived N accumulation [shoot or fruit dry weight biomass (mg) × total N concentration (%) × %RdN/10⁴ (data not shown)] between a given week and the preceding week. Likewise, total N uptake was calculated as the positive difference of the shoot or fruit total N accumulation [shoot or fruit dry weight (mg) × N concentration (%)/100 (data not shown)] between a given week and the preceding week.

Statistical analysis

The significance of mean differences among treatments was tested using one-way ANOVA and Tukey's honestly significant difference test in R software version 3.5.3 (R Core Team, 2019). For evaluating the significance of differences between two mean values, the *t*-test was used. Differences were accepted as significant if $P \le 0.05$ unless otherwise stated.

Results and Discussion

Soil inorganic nitrogen

The incorporation of cover crop residues in the soil significantly influenced soil inorganic N (SIN) (NH₄⁺⁻ N + NO₃⁻⁻N) levels at 1, 2, and 4 WAT only (Fig. 1). The SIN levels were greater in cover crop treatments, especially HV+RYE (323 mg N/plant) and HV (295 mg N/plant), than in BARE (252 mg N/plant) at 1 WAT. At 2 WAT, HV showed the highest SIN levels (259 mg N/plant) followed by HV+RYE (176 mg N/plant), whereas RYE (98.1 mg N/plant) and BARE (106 mg N/plant) showed the lowest SIN levels. The HV treatment also showed the highest SIN levels (101 mg N/plant) at 4 WAT followed by HV+RYE (57.9 mg N/plant) and RYE (30 mg N/plant), whereas BARE (15.1 mg N/

plant) showed the lowest SIN levels.

Averaged across sampling dates, SIN levels followed this order: HV > HV+RYE > RYE > BARE (data not shown). Higher SIN levels with cover crops than with no cover crops may be the result of higher N input by hairy vetch and rye residues with a C/N ratio < 25 (Allison, 1966) (Table 1). The fact that SIN levels increased in HV treatment more than in RYE treatment, despite a similar N input, may be explained by faster decomposition of hairy vetch residues after incorporation in the soil because of the high N concentration and low C/N ratio (Frankenberger and Abdelmagid, 1985). Overall, SIN levels tended to decrease in all treatments from 1 to 12 WAT, possibly due to the increase in tomato N demand with growth.

Tomato shoot biomass and fruit yield

The effect of cover crop residues on shoot biomass was significant at 8 and 12 WAT only (Table 2). RYE, HV, and HV+RYE showed similar and greater shoot biomass than BARE at 8 and 12 WAT. The fruit dry weight yield was significantly greater in HV and HV



Fig. 1. Effects of hairy vetch and rye on inorganic nitrogen (NO₃⁻⁻ N + NH₄⁺-N) in the pot soil. Means followed by the same letters in each week are not significantly different at 5% by Tukey's honestly significant difference test. NS, not significant. Vertical bars represent standard errors; only shown when larger than the symbols (n = 5).

+RYE than in RYE and BARE (Table 2).

Greater shoot biomass and fruit yield in HV and HV +RYE than in BARE may have resulted from more soil N availability in cover crop treatments (Fig. 1). Greater tomato growth and yield with hairy vetch than with bare fallow have been reported by other researchers (Araki et al., 2009; Sainju et al., 2000, 2003). As opposed to hairy vetch, rye is known to have little or no effect on soil N availability and crop yield because of low N concentration and high C/N ratio (Kuo et al., 2001; Sainju et al., 2005). In the present study, because of a C/N ratio < 25 of rye residues (Allison, 1966) [(C/N = 17.4) (Table 1)], the RYE treatment led to higher SIN levels than the BARE treatment at 1 and 4 WAT (Fig. 1), whereas such an increase in soil N availability had no effect on fruit yield (Table 2). These results suggest that regardless of its C/N ratio, rye monoculture may not be effective for increasing tomato fruit yield; therefore, the use of the biculture of hairy vetch and rve may represent a better option to increase fresh-market tomato yield.

Total nitrogen uptake

Total N uptake by tomato shoot varied significantly with treatments at 2–4 and 4–8 WAT only (Table 3).

 Table 2. Tomato shoot biomass and fruit yield as influenced by the incorporation of cover crop residues in pots.

	Sho	Fruit drv			
Treatments		weight			
	2	4	8	12	(g DW/plant)
BARE	8.80	22.7	44.6 b ^z	49.4 b	93.2 b
HV	8.00	17.4	81.4 a	89.4 a	132 a
RYE	9.20	17.4	72.6 a	84.4 a	97.6 b
HV+RYE	9.40	17.3	77.2 a	97.6 a	130 a
Significance	NS	NS			

^z Means followed by the same letters in each column are not significantly different at 5% by Tukey's honestly significant difference test. NS, not significant.

Table 3. Effects of rye and hairy vetch on total N uptake by tomato shoot and fruit.

	N uptake (mg N/plant)								
T ()		Sho	oot			Fi	ruit		
Treatments	WAT				WAT			T (1	Grand total
	0–2	2–4	48	Total	0–2	2–4	4-8	- Iotal	
BARE	282	130 b ^z		412 c	84.1	166	370 b	620 c	1032 c
HV	241	347 a	324 a	912 a	99.9	173	908 a	1182 a	2093 a
RYE	260	215 ab	160 b	635 b	72.8	134	780 a	987 b	1622 b
HV+RYE	290	257 ab	157 b	704 b	104	184	921 a	1210 a	1914 a
Significance	NS	Ť			NS	NS			

^z Means followed by the same letters in each column are not significantly different at 5% or 10% by Tukey's honestly significant difference test.

[†] Significant at P<0.1.

NS, Not significant.

denotes no N uptake.

Shoot N uptake was greater in cover crop treatments, especially HV, than in BARE at 2–4 WAT. As opposed to 2–4 WAT, shoot N uptake was observed in cover crop treatments only at 4–8 WAT, and HV showed greater shoot N uptake than RYE and HV+RYE. Shoot N uptake from transplanting to 8 WAT increased in HV more than in HV+RYE and RYE, compared with BARE. The greater shoot N uptake in cover crop treatments than in no cover crop treatment resulted from higher soil N availability in the cover crop treatments, especially in HV (Fig. 1). The cessation of shoot N uptake in BARE just after 4 WAT may be the result of N partitioning to fruit, a process that results from low soil N availability (Cambui et al., 2011; de Groot et al., 2003).

As opposed to shoot N uptake, the effects of cover crops on fruit N uptake were significant at 4-8 WAT only, and the cover crop treatments showed similar and greater fruit N uptake than BARE treatment (Table 3). Fruit N uptake from transplanting to 8 WAT increased in HV and HV+RYE more than in RYE, compared with BARE. The fact that fruit N uptake ceased after 8 WAT in all treatments may be explained by low soil N availability after 8 WAT (Fig. 1) and suggests that N applied by cover crop residues was insufficient to sustain tomato growth for a longer period than 8 WAT. Overall, HV and HV+RYE contributed more N to shoot growth and fruit production than RYE and BARE (Table 3), and this may explain the greater fruit yields in HV and HV +RYE than in RYE and BARE. RYE contributed less N because of a higher C/N ratio of residues [(C/N ratio of residues in HV, HV+RYE, and RYE was 9.69, 13.6, and 17.4, respectively (Table 1)] (Frankenberger and Abdelmagid, 1985).

The proportion of total N in tomato shoot and fruit derived from rye

The proportion of total N in the shoot and fruit derived from rye (%RdN) was significantly greater in RYE treatment than in HV+RYE treatment on all sampling dates because of higher N input by rye residues in RYE treatment (Fig. 2). The %RdN in the shoot decreased in both treatments from 2 to 8 WAT, but it decreased in RYE treatment (43 to 32%) more than in HV +RYE treatment (21.4 to 17.2%); no marked change in %RdN in the shoot was observed after 8 WAT (Fig. 2A). Likewise, %RdN in fruit decreased in both treatments with time, but marked changes in %RdN were observed in RYE treatment at 2 to 4 WAT (52.7 to 38.8%) and 8 to 12 WAT (39.3 to 31.9%) (Fig. 2B). The decrease in %RdN in tomato shoot and fruit in both treatments after 2 WAT suggests that rye-derived N availability in the soil decreased with time, possibly the result of an increase in tomato N demand with growth.

The fact that %RdN in shoot and fruit decreased in RYE treatment more than in HV+RYE treatment suggests that rye residues in HV+RYE treatment may have contributed more N, in relation to the N input, than rye



Fig. 2. The proportion of the total N in tomato (A) shoot and (B) fruit derived from rye as influenced by residue management. Vertical bars represent standard errors; only shown when larger than the symbols (n = 5). ***Significant at P < 0.001.

residues in RYE treatment. The decrease in %RdN in shoot and fruit after 2 WAT agreed with the results of Sugihara et al. (2013), who reported a decrease in the proportion of total N in tomato derived from hairy vetch after 2 WAT. These similar results suggest that the decrease in the proportion of total N in tomato biomass derived from cover crop residues after 2 WAT may be more related to the increase in tomato N demand with growth rather than the nature of plant residues applied to the soil.

Rye-derived nitrogen uptake

Rye-derived N uptake by tomato shoot was significantly greater in RYE than in HV+RYE on all sampling dates (Table 4). However, rye-derived N uptake by tomato fruit was significantly greater in RYE than in HV+RYE at 0-2 WAT and 4-8 WAT only. Rye-derived N uptake by tomatoes (shoot + fruit) from transplanting to 8 WAT represented 32.2% and 17.3% of the total N uptake in RYE (1622 mg N/plant) and HV+RYE (1914 mg N/plant), respectively (Table 4). Because of higher rye-derived N input in the soil, RYE treatment contributed more rye-derived N to shoot growth and fruit production than HV+RYE treatment. Rye-derived N uptake by tomato shoot and fruit ceased at 4-8 WAT in both treatments, possibly the result of depletion of rye-derived inorganic N in the soil. HV+RYE showed high rye-derived N uptake by tomatoes (shoot + fruit) in the early (0-4 WAT) and late (4-8 WAT) periods of tomato cultivation. High rye-derived N supply in the late period is very important because tomatoes require high amounts of N for fruit production during that period (Hartz and Bottoms, 2009).

It's noteworthy that because of N fertilization with ¹⁵NH₄Cl and high initial soil inorganic N (84.1 mg N·kg⁻¹), the C/N ratio of rye in this study was lower than the normal range reported in many studies [25 to 49 (Clark et al., 1994; Kuo et al., 1997a, b; Sainju et al., 2003, 2005)], therefore, N release from rye residues (C/N ratio \geq 25) in the early period of tomato cultivation may not be as high as observed in this study because high C/N ratio of residues increases the N immobilization potential and delays N release (Frankenberger and Abdelmagid, 1985; Sainju et al., 1998).

Rye-derived nitrogen recovery efficiency

The rye-derived N recovery by tomato shoot was significantly greater in HV+RYE treatment than in RYE treatment at 0–2 and 2–4 WAT, whereas the RYE treatment showed greater rye-derived N recovery by tomato shoot than HV+RYE treatment at 4–8 WAT (Table 5). Rye-derived N recovery by tomato fruit was significantly greater in HV+RYE than in RYE at 0–2 and 4– 8 WAT only. The total rye-derived N recovery by tomatoes (shoot + fruit) from transplanting to 8 WAT was 26.9% in RYE treatment and 34.0% in HV+RYE treatment (Table 5). These results suggest that the biculture may be an effective management practice for increasing N recovery from rye or hairy vetch + rye by tomatoes; therefore, high N fertilization rates that have been associated with N leaching to groundwater (Meisinger et al., 1991) can be avoided.

Despite the positive effect of hairy vetch on crop yields (Clark et al., 1994; Sainju et al., 2003, 2005), a large amount of applied N from hairy vetch is not recovered by tomatoes (Muchanga et al., 2019; Sainju et al., 1999); a portion of unrecovered N by tomatoes is retained by the soil in organic form (Sainju et al., 2003), and the inorganic N portion is likely to be lost from the soil-plant system. Sainju et al. (1999) reported higher residual nitrate levels in the soil and nitrate leaching to groundwater in tomato production with hairy vetch than with bare fallow. Similarly, Kuo et al. (2001) reported higher nitrate leaching to groundwater in corn production with hairy vetch than either rye or the biculture of hairy vetch and rye. Therefore, the biculture of hairy vetch and rye may represent a better option to reduce nitrate leaching to groundwater while increasing tomato yield.

Studies on ¹⁵N-labeled nonlegume recovery by subsequent crops, especially vegetables, are very limited. Muller and Sundman (1988) reported N recovery by barley (*Hordeum vulgare* L.) of 22% from timothy grass (*Phleum pratense* L.). N recovery by barley from legumes (red clover, subterranean clover, and white clover) ranged from 17 to 24%. This suggests that irrespective of the quality of cover crop residues (legume or nonlegume) added to the soil, plant-derived N recovery by a subsequent crop may be low; thus, the biculture of legume and nonlegume may be a better

	Rye-derived N uptake (mg N/plant)									
Treatments -		Sh	oot		Fruit					Percentage
		WAT		T (1		WAT		Grand total		N uptake ^z
	0–2	2–4	4–8	Total	0–2	2–4	4-8	- Iotai		1
RYE	112	67.2	25.4	205	31.4	46.9	240	318	523	32.2
HV+RYE	62.0	50.6	9.13	122	22.3	44.3	142	209	331	17.3
t-test	*	Ť	**	***	Ť	NS	**	***	***	_

Table 4. Rye-derived nitrogen uptake by tomatoes as influenced by residue management.

^z Calculated as the ratio of rye-derived N uptake (shoot+fruit) to the total N uptake (1622 and 1914 mg N/plant for RYE and HV+RYE treatments, respectively).

⁺,*,**,*** Significant at P<0.1, 0.05, 0.01, and 0.001, respectively.

NS, not significant.

Table 5.	Rye-derived nitrogen re	covery by tomatoes	as influenced by	residue management.
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				Rye-deri	ved N recov	very (%)			
Treatments		Sh	oot		Fruit				
		WAT		T- 4-1	WAT			T- 4-1	Grand total
	0–2	2–4	4-8	Total	0–2	2–4	4-8	— Iotai	totui
RYE	5.77	3.46	1.31	10.5	1.61	2.41	12.4	16.4	26.9
HV+RYE	6.38	5.21	0.94	12.5	2.30	4.56	14.6	21.5	34.0
<i>t</i> -test	*	t	**	***	Ť	NS	**	***	***

 ,*,**,*** Significant at P < 0.1, 0.05, 0.01, and 0.001, respectively. NS, not significant.

management practice to increase plant-derived N recovery by subsequent crops, especially vegetables, which are known to recover less N from plant residues and fertilizers than cereal crops (Di and Cameron, 2002).

In this study, the higher rye-derived N recovery in HV+RYE than in RYE may not fully explain the greater fruit yield in HV+RYE than in RYE because ryederived N uptake (shoot + fruit) in RYE was greater than that in HV+RYE. The greater N contribution of both hairy vetch and rye in HV+RYE treatment to fruit production compared with that of rye residues in RYE treatment (Table 3) may be the major reason for the greater yield in HV+RYE than in RYE. Although the N contribution of rye residues to shoot growth and fruit production in HV+RYE treatment increased compared with that in RYE treatment, the data in Table 6 suggest that hairy vetch residues in the biculture treatment contributed more N to shoot growth and fruit production than rye residues, especially at 4-8 WAT. Hairy vetch and rye N contributions from transplanting to 8 WAT represented 25.3% and 17.3% of the total N uptake (1914 mg N/plant), respectively (Table 6). In a previous study, N recovery from hairy vetch (monoculture) by tomatoes grown in pots was estimated at 40.3% by 4 WAT and only 15% after that period (N uptake ceased after 10 WAT) (Sugihara et al., 2013). Thus, the results of this study suggest that the biculture may change the N release pattern from both hairy vetch and rye. Hairy vetch, when mixed with rye, may contribute more N to shoot growth and fruit production after 4 WAT (reproductive stage) than during the first 4 WAT (vegetative stage). In turn, rye may contribute more N when mixed with hairy vetch than when used alone. Because the N contribution of hairy vetch to shoot growth and fruit production was estimated while considering that soil N contribution in RYE and HV+RYE treatments was similar, a further study examining the N uptake and recovery by tomatoes from ¹⁵N-labeled hairy vetch residues mixed with rye residues may be necessary to clarify the role of hairy vetch in the biculture management.

The data in Table 6 also suggest that hairy vetch and rye combined may supply large amounts of N in the

early (0–4 WAT) and late (4–8 WAT) periods of tomato cultivation. This may be the major reason for the greater yield and residue-derived N recovery in the biculture of hairy vetch and rye (marketable yield and N recovery were 134 t·ha⁻¹ and 63.9%, respectively) than in the hairy vetch monoculture (fast N release) (marketable yield and N recovery were 116 t·ha⁻¹ and 42%, respectively) observed in our previous study (Muchanga et al., 2020).



The rye-derived N levels in the soil varied signifi-





Components of the biculture		WAT	T-4-1	Percentage of total N uptake ^z		
	0–2	2–4	4-8	Total	total it aparte	
Hairy vetchy	120	111	253	484	25.3	
Rye	84.3	94.9	151	331	17.3	
<i>t</i> -test	**	NS	**	***	_	

Table 6. Nitrogen uptake by tomatoes (shoot + fruit) from each cover crop in the biculture.

z Calculated as the ratio of N uptake from hairy vetch or rye to the total N uptake of the biculture treatment (1914 mg N/plant).

^y N uptake from hairy vetch was calculated as the difference of N uptake from the soil+hairy vetch in HV+RYE treatment and N uptake from the soil only in RYE treatment, considering that the amount of N uptake from the soil by tomatoes in RYE and HV+RYE treatments was similar (soil or soil+hairy vetch N uptake was calculated as the difference of total N uptake and rye-derived N uptake in RYE or HV+RYE).

NS, not significant.

^{**,***} Significant at P<0.01 and 0.001, respectively.

cantly with residue management on all sampling dates (Fig. 3A). The RYE treatment showed markedly higher rye-derived N levels in the soil on all sampling dates (866, 799, 836, and 1021 mg N/pot at 2, 4, 8, and 12 WAT, respectively) than HV+RYE treatment (542, 503, 454, and 457 mg N/pot at 2, 4, 8, and 12 WAT respectively). Greater rye-derived N retention in the soil in RYE treatment than HV+RYE treatment may be, in part, related to a greater amount of rye residues applied to the soil in RYE than in HV+RYE (Kuo et al., 1997b), but also to higher rates of rye-derived N mineralization in HV+RYE than in RYE. The fact that soil rye-derived N levels in RYE treatment tended to increase from 2 to 12 WAT, but those in HV+RYE treatment tended to decrease in a similar period (Fig. 3A), suggests that slow decomposition may be more advantageous than fast decomposition of rye residues to build up soil total N. The proportion of soil total N derived from rye varied positively from 4.9 to 7.0% (2 to 12 WAT) in RYE treatment and negatively from 3.4 to 3.1% in HV+RYE treatment (Fig. 3B).

The low rye-derived N recovery by tomatoes found in this study was the result of high soil N retention. At 12 WAT, the amount of rye-derived N retained in the soil represented 52.5% in RYE and 47.0% in HV+RYE of the rye-derived N input in each treatment [972 and 1943 mg N/pot in HV+RYE and RYE, respectively (Table 1)]. The amount of rye-derived N in tomato roots and possibly lost (the difference of rye-derived N input and rye-derived N retained in the soil and in the tomato shoot and fruit) was estimated at 19.0% and 20.6% of the rye-derived N input in HV+RYE and RYE treatments, respectively. However, N losses may have been negligible as the irrigation was managed to avoid N leaching and weeds that appeared were shredded and returned to the same pots. The ability of the soil to retain plant-derived N is stronger compared with the ability of the subsequent crop and different loss mechanisms to remove it (Muller and Sundman, 1988). Other studies also reported greater increases in plantderived N retention by the soil than losses through leaching or denitrification (Cueto-Wong et al., 2001a, b; Muller and Sundman, 1988; Seo et al., 2006).

In conclusion, our research hypotheses were mostly supported by the results. However, rye-derived N uptake by tomatoes in the biculture treatment was not greater than that in rye monoculture treatment, as hypothesized. Overall, the results showed that the N contribution of rye to tomato growth and fruit production may be increased by a combined application with hairy vetch in the soil. However, hairy vetch in the biculture may contribute more N than rye to tomato production, and the N contribution of hairy vetch may be more significant after 4 weeks following the transplanting of tomatoes (reproductive stage) than during the first 4 weeks after transplanting (vegetative stage). Therefore, the biculture may change the N release pattern of both hairy vetch and rye, with the cover crops releasing large amounts of N in both the early and late periods of tomato cultivation. The results of this study may help improve N management in vegetable production systems by maximizing the use of plant-derived N by crops, thereby reducing N fertilizer inputs.

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