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Abstract: Plant-induced solubilization of soil nitrogen (N) is a key process for plants to utilize the recalcitrant form of N. To evaluate its contribution to plant uptake, the factors affecting the contribution and the forms of N solubilized by plants, we analyzed the results of a pot experiment in which komatsuna (*Brassica rapa* L. var. *peruviridis*) was grown in 3 different soils applied with 5 types of sewage sludge as a source of N for plants. The amount of N solubilized by plants, which was defined experimentally as the difference in the amount of solubilized N between the planted and unplanted treatments, varied with the soil types more than the types of sewage sludge. This accounted for 30% (Arenosol), 15% (Fluvisol) and 1.6% (Andosol) of the amount of N uptake on average. These percentages were high when the level of soil soluble N after the experiment was below approximately 30 mg kg^{-1}. Sequential analyses of insoluble N in soil after the experiment indicated the occurrence of plant-induced solubilization of both bio-soluble and acid-soluble N in many of the Arenosol and Fluvisol treatments and that of acid-soluble N in the Andosol treatments. The plant-induced solubilization in the Andosol resulted in the accumulation of more labile bio-soluble N rather than enhanced plant uptake. For komatsuna grown in fertilized soil, the depletion of soluble N in the root zone seems to be important for the increase in the contribution of the plant-induced solubilization to uptake but not for the occurrence of the solubilization.

Key words: Availability, Komatsuna (*Brassica rapa* L. var. *peruviridis*), Plant-induced solubilization, Sewage sludge, Soil nitrogen.

Of all the nutrients supplied from soil, nitrogen (N) is the most important nutrient for plant growth. Until recently, the amount of mineralized N evaluated by the incubation method had been regarded as the most reliable estimate of the amount of N available to plants. This estimation would be fully satisfactory when roots act as only a sink of N supplied from soil by mass flow and diffusion. However, the amount of N available to plants may vary considerably with the plant species even when plants are grown in small pots in order to minimize the effect of root foraging capacity (Hayashi and Harada, 1964; Matsumoto et al., 1999). This is partly because a number of species especially those growing in N-limited environments have developed special strategies to increase the forms of available N. In such cases, measurements of mineralizable N without growing plants may underestimate the amount of plant-available N (Jonasson et al., 2006).

The above strategies enable the plants to capture additional N from soil by solubilization, from the air by symbiotic fixation and even from insects by preying. Among them, the solubilization of N by plants, which is defined as the plant-induced process releasing insoluble N from the soil solid phase to the solution phase, has prevailed most widely (Schimel and Bennett, 2004). As shown in Fig. 1, the solubilization is the first key process for both plants and microbes to utilize N in the soil solid phase. Microbes are the main driver of this process, but some plant species can affect it significantly through the large supply of carbon from root to the surrounding microbes (Frank and Groffman, 2009). The primary reaction involved in the plant-induced solubilization is desorption of organic N compounds from the solid phase by extracellular enzymes released from microbes (Weintraub and Schimel, 2005). The solubilized organic N is subsequently taken up by plants through enhanced N mineralization (in the case of absorption of inorganic N after mineralization of the solubilized N) and direct uptake of organic N (in the case of absorption of the soluble
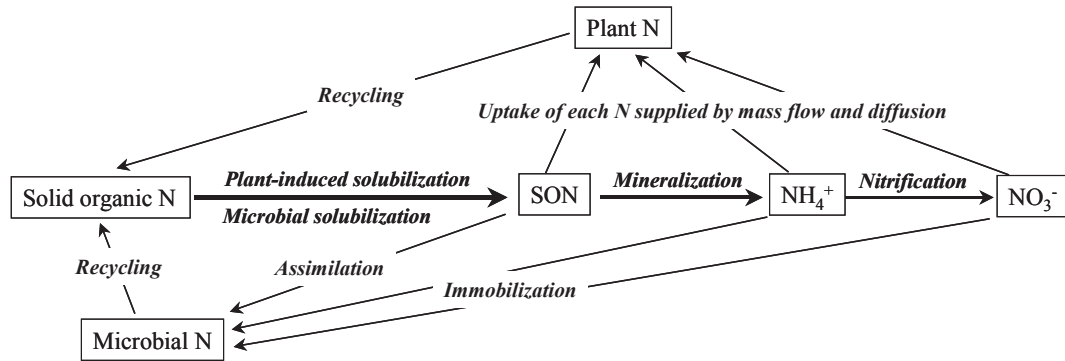
Fig. 1. Dynamics of N in soil with plant-driven processes. In the figure, solubilization is considered as a prerequisite for mineralization by referring to the soil N cycle proposed by Schimel and Bennett (2004). The width of the arrows indicates the relative possible magnitude of the flows. SON: soluble organic N extractable with salt solutions such as KCl, CaCl$_2$ or K$_2$SO$_4$.

organic N or its partially decomposed components before mineralization). Desorption of fixed NH$_4^+$ from the 2:1 type clay minerals is also one of the solubilization processes (Scherer and Werner, 1996).

Ample evidence has shown the occurrence of enhanced mineralization (e.g. Hayashi and Harada, 1964; Parkin et al., 2002; Colin-Belgrand et al., 2003) and of direct uptake of soluble organic N (e.g. Xu et al., 2006; Jones et al., 2005; Näsholm et al., 2009). Furthermore, Miyazawa et al. (2010), who confirmed that Qing-geng-cai (*Brassica chinensis* L.) grown under aseptic condition can barely utilize high molecular weight organic N in a soil extract with a neutral phosphate-buffer solution, pointed out the importance of examining the translocation and utilization of soluble organic N by plants following its direct uptake by roots.

On the other hand, plant-induced solubilization of soil N that can trigger these processes has not been fully investigated. In particular, information is still lacking on the contribution of such solubilization to the total amount of N taken up by plants. The high contribution has been reported only in a few plant species such as sorghum (*Sorghum bicolor* L.) (Okamoto and Okada, 2004), white lupin (*Lupinus albus* L.) (Watanabe et al., 2006) and a viviparous grass in the sub-arctic montane (*Festuca vivipara* L.) (Jonasson et al., 2006). Further confirmation of these results is required to estimate the importance of plant-induced solubilization of soil N as one of the N-driving processes in the soil-plant systems (Fig. 1).

Komatsuna (*Brassica rapa* L. var. *peruviridis*) is a leafy vegetable originating in Japan. It is one of the most popular vegetables in this country and has been used by many researchers. According to Matsumoto and Ae (2004), this plant is likely to have high ability to solubilize N in the soil solid phase. In their experiment, komatsuna was grown in 3 different soils for 30 days after application of fertilizers except N. The content of neutral phosphate-buffer extractable organic N in soil around roots became much lower than the content in soil before the experiment when

the plant was grown in a Regosol. By contrast, the content of N in this form became higher in the soil around the roots when the plant was grown in an Andosol or a Fluvisol. The authors considered from these results that komatsuna must have removed and utilized phosphate-buffer extractable organic N when it was grown in the Regosol and that it may have replenished this N with less extractable organic N when it was grown in the other soils having greater reserves of stable organic N.

However, it is unknown how much the ability of komatsuna to solubilize N contributes to plant uptake especially when the plant is grown in heavily fertilized soils distributed widely in Japan. Using komatsuna as a test plant, we aimed to evaluate the contribution of plant-induced solubilization to the amount of N taken up by plants, the factors affecting the contribution and the forms of N solubilized by plants.

Materials and Methods

1. Pot experiment

Part of the experimental results to be analyzed here overlaps with those of our previous pot experiment in which komatsuna plants were grown in three soils applied with sewage sludge as a source of N for plants (Moritsuka et al., 2006). Sewage sludge is a by-product of wastewater treatment that releases inorganic N at a relatively high rate and also increases the reserves of organic N in soil. Effects of application of sewage sludge on plant growth and soil solution dynamics are described in Moritsuka et al. (2006).

Three types of soil materials (Andosol, Fluvisol and Arenosol) were sampled from the surface of agricultural fields in Japan, and subjected to air-drying and 2-mm mesh sieving. Sewage sludge derived from a wastewater treatment plant in Shimane prefecture, Japan was used as N fertilizer. Because the heating treatment at different temperature and moisture conditions can change the rate of N mineralization (Matsuoka et al., 2006), we prepared 5 types of sludge materials from the above sewage sludge by

Table 1. Selected properties of the soils and sewage sludge used in this experiment.

		Total C[a]	Total N[a]	Total P[b]	Inorganic N	Mineralizable N[c]	Sand	Silt	Clay
		—————(g kg⁻¹)—————			—————(mg kg⁻¹)—————		—————(g kg⁻¹)—————		
Soil	Andosol	60.8	4.8	1.4	69	150	678	202	121
	Fluvisol	13.9	1.4	2.0	18	64	729	148	123
	Arenosol	3.7	0.4	0.4	4	15	984	6	10
Sludge	AD	364.4	60.6	25.9	1732	18247	ND	ND	ND
	AD-120	370.8	61.8	22.2	1625	24104	ND	ND	ND
	AD-180	391.1	67.9	24.2	785	8372	ND	ND	ND
	HD-120	374.0	62.3	22.7	3656	18858	ND	ND	ND
	HD-180	380.8	63.6	24.1	2620	16073	ND	ND	ND

Data cited from Matsuoka et al. (2006).

[a]Measured by the dry combustion method using a CN corder (Yanaco, MT-700).

[b]Measured by the vanadomolybdophosphoris acid method after wet digestion with H_2SO_4, HNO_3 and $HClO_4$ (Kuo, 1996).

[c]Amount of N mineralized during the 84-day aerobic incubation period at 30°C (Ogawa, 1997).

ND: Not determined.

the following treatments; air-drying (AD), dry-heating at 120°C (AD-120), dry-heating at 180°C (AD-180), heat-drying at 120°C (HD-120), and heat-drying at 180°C (HD-180). Dry-heating described here indicates that the sludge containing 89% water was air-dried and then heated, whereas heat-drying indicates that the sludge containing water was heated without air-drying. These products were crushed to pass through a 1-mm mesh sieve. As shown in Table 1, the contents of inorganic and mineralizable N varied considerably among soils and sludge materials.

Samples of the above soils were put in 1/5000-a Wagner pots at the rate of 3.0 kg (Andosol), 4.0 kg (Fluvisol), and 4.6 kg (Arenosol) per pot. The pots had a closed bottom. The above sludge materials were added to each soil sample at the rate of 40 g dry matter weight per pot (equivalent to 20 Mg ha⁻¹), and the sludge and soil were mixed homogeneously. Distilled water was then poured onto the surface of each pot to make the soil moisture content of 60% of the maximum water-holding capacity. Half a day after watering, about 12 seeds of komatsuna were sown in each pot. Unplanted pots were also prepared. The planted pots were triplicated and the unplanted pots were duplicated. Before and during the experiment, fertilizers other than the sludge materials were not applied.

The experiment was conducted in a greenhouse at Shimane University for 88 days from November 22, 2003 to February 18, 2004. During the experiment, the soil moisture content was maintained at the initial level every few days by measuring the decrease in weight of the planted and unplanted pots and by adding the necessary amount of distilled water to each pot. On day 21, the seedlings were thinned to three. The experiment was conducted until some of the old leaves turned yellow, displaying symptoms of N deficiency.

2. Sampling and analysis of plants and soils

After the pot experiment, plant shoots were harvested. Roots were also sampled after removal of adhering soils by shaking and washing in distilled water. To sample the remaining roots, the soil in the planted pots was totally passed through a 2-mm mesh sieve and the roots on the sieve were collected. The plant samples were oven-dried at 70°C and weighed. The concentration of N was determined by the dry combustion method (Sumigraph NC-80 auto, Sumika Chem. Anal. Service, Osaka, Japan).

Soil was also sampled from each pot after complete homogenization by air-drying and 2-mm mesh sieving. The total volume of the soil sample was less than 50 g per pot. To extract soluble N, 5 g of the samples was placed in a 50 mL centrifuge tube and shaken reciprocally at room temperature for 30 min after addition of 25 mL of 0.5 M K_2SO_4. Then, the extracts were obtained by centrifugation at 2000 rpm for 10 min and filtration. The residual soils were extracted again with 25 mL of distilled water. The extracts were added to the former ones and the total volume was filled up to 50 mL. The concentration of soluble N in the extracts was determined by the colorimetric method after persulfate digestion. For the extraction of soluble N in soil, we used a potassium sulfate solution instead of a more conventional potassium chloride solution, because chloride ion competes with sulfate radicals and prevents the mineralization of dissolved organic N during the persulfate digestion (Peyton, 1993).

The residual insoluble N, which is in organic form except for interlayer NH_4^+, was further subjected to the analysis of solubilizable N.

First, the amount of N solubilized by microbes was determined by measuring the amount of soluble N increased by soil incubation for 14 days at 30°C. The

incubation was conducted by placing 5 g of sample in a 50 mL centrifuge tube and maintaining the moisture content at 60% of the maximum water holding capacity during the incubation period. After the incubation, K_2SO_4-soluble N was extracted and the concentration was determined by the methods described above. Hereafter, the form of N solubilized by this biological treatment is referred to as 'bio-soluble' form.

Second, the amount of N solubilized by strong acidification was determined by applying acid extraction to the soils remained after the above incubation whose N in both soluble and bio-soluble forms had been removed by the K_2SO_4 extraction. By referring to the method used by Ito and Ae (2000), the residual soils were shaken reciprocally at 25°C for 2 h after addition of 25 mL of 0.1 M H_2SO_4, and the extracts were obtained by centrifugation at 2000 rpm for 10 min and filtration. The residual soils were extracted again with 25 mL of distilled water. The extracts were added to the former ones and the total volume was filled up to 50 mL. The concentration of N in the extracts was determined using a total organic carbon analyzer equipped with TNM-1 (Shimadzu, TOC-V, Kyoto, Japan). Hereafter, the form of N solubilized by this chemical treatment is referred to as 'acid-soluble' form.

3. Calculation of amount of N supplied by plant-induced solubilization

From the above soil analyses, the amounts of K_2SO_4-soluble N remaining in either planted or unplanted soil were calculated by multiplying the initial soil weight per pot by the content of soluble N. The amount of N taken up by the plants was also calculated by subtracting the content of N in the seeds from that in the plants including the roots.

Here we define plant-induced solubilization experimentally as the plant-induced process releasing K_2SO_4-insoluble N (such as bio-soluble and acid-soluble N) from the soil solid phase to the solution phase, so that we can estimate its magnitude from the results obtained.

The amount of N solubilized from soil in the presence of plants (Sol-plant) can be expressed as follows.

(Sol-plant) = (Amount of plant N uptake) + (Amount of soluble N remaining in planted soil)−(Amount of soluble N in soil before experiment)

On the other hand, the amount of N solubilized from soil in the absence of plants (Sol-control) can be expressed as follows.

(Sol-control)=(Amount of soluble N remaining in unplanted soil)−(Amount of soluble N in soil before experiment)

Since the amount of plant-induced solubilization is the difference between Sol-plant and Sol-control, it can be expressed as follows; (Amount of plant N uptake)+ (Amount of soluble N remaining in planted soil)−

(Amount of soluble N remaining in unplanted soil).

It should be noted here that the above calculation does not account for the N flows between soil and the air (such as denitrification and ammonia volatilization), and assumes that such flows were not affected by the presence of plants.

Results

1. Contribution of plant-induced solubilization of N to its uptake

The dry matter weight of plants varied from 10.0 to 24.5 g pot^{-1}, depending on soil and sludge treatments (Table 2). Since plant growth in the Andosol treatments was not restricted by N but limited by phosphorus (P) due to intensive fixation of soluble P by both the soil and the applied sludge (Moritsuka et al., 2006), the dry matter weight of plants was relatively lower than that of the plants grown in other soils despite the highest content of inorganic and mineralizable N in the Andosol (Table 1).

In accordance with plant growth and N uptake, the amount of soluble N remaining in soil became much lower than the unplanted control (Table 2). The sum of the N uptake and remaining soluble N in the planted soil exceeded the amount of remaining soluble N in the unplanted control especially in the Arenosol treatments. This indicated the presence of plant-induced solubilization. The amount of N solubilized by plants varied with soil type. The average contribution of this process to N uptake was the highest in the Arenosol (30%) followed by the Fluvisol (15%) and then the Andosol (1.6%). The effect of type of sewage sludge on the percentage of the contribution was not as conspicuous as that of soil type.

A critical factor affecting the contribution of plant-induced solubilization seems to be the content of soluble N in the root zone. In Fig. 2, the content of K_2SO_4-soluble N remaining in the planted soil after the experiment is plotted against the contribution of plant-induced solubilization to N uptake. The contribution was high when the level of soluble N after the experiment was below approximately 30 mg kg^{-1}. The soluble N content was affected more by soil type than by sludge type, which explains why the contribution varied with the soil treatments more widely than with the sludge treatments.

2. Plant-induced solubilization indicated by the analysis of N forms in soil

In the Andosol treatments, plant growth was not limited by the soluble N supply. The amount of N solubilization reached only a few percent of the amount of N uptake (Table 2). This suggests that the depletion of soluble N in soil was caused mostly by plant uptake. It is therefore likely that the magnitude of plant N uptake was much higher than that of other N flows (denitrification and ammonia volatilization) that were not accounted for in the

Table 2. Dry matter weight of komatsuna plants, and amounts of N uptake, remaining soluble N in soils and N supplied by plant-induced solubilization.

		Dry matter weight of plants	N uptake	Soluble N remained in planted soil	N uptake plus soluble N remained in planted soil	Soluble N remained in unplanted soil	Amount of plant-induced solubilization	Contribution of plant-induced solubilization
		(g pot^{-1})			(mg pot^{-1})			(% to N uptake)
Andosol	AD	13.5±0.24	618±20	143±15	762±18	739±50	23	4
	AD-120	13.9±0.14	659±18	253±7	911±16	897±51	14	2
	AD-180	12.4±0.18	507±10	98±3	605±13	600±44	6	1
	HD-120	11.5±1.00	521±24	456±12	977±13	972±52	5	1
	HD-180	13.8±1.03	620±27	247±23	867±5	884±19	–17	0
Fluvisol	AD	23.4±0.36	580±8	63±5	642±3**	543±19	100	17
	AD-120	23.7±0.60	726±6	76±4	802±6	732±69	70	10
	AD-180	17.8±0.55	381±3	94±6	475±9	408±41	68	18
	HD-120	24.5±1.34	805±9	109±4	914±8	804±81	110	14
	HD-180	22.8±1.53	669±6	102±2	771±8**	645±13	126	19
Arenosol	AD	16.4±1.36	423±15	26±13	449±3*	322±44	127	30
	AD-120	17.1±0.95	610±8	36±18	646±24**	451±1	195	32
	AD-180	10.0±0.54	223±3	12±2	235±5**	158±14	77	35
	HD-120	16.1±1.15	692±16	39±5	730±11*	564±35	166	24
	HD-180	14.4±1.13	511±11	60±4	571±9*	428±35	142	28

Part of data cited from Moritsuka et al. (2006).

Values are average±standard errors of three replicates for planted treatment and two replicates for unplanted treatment.

Values with ** and * indicate significant differences from the corresponding unplanted control (right next column) at the level of P<0.01 and P<0.05, respectively (t-test).

The amount of plant-induced solubilization in the HD-180 (Andosol) treatment became negative probably due to an experimental error and was taken as zero in Fig. 2.

calculation. However, it should be noted that the amount of N solubilized by plants presented in Table 2 was calculated indirectly from the difference between the amount of N taken up by plants and the amount of soluble N decreased by plants. If the solubilized N had not been absorbed by plants but returned to the soil solid phase as insoluble form with an aid of microbial immobilization (a pathway depicted in the lower part of Fig. 1), the presence of such solubilization could not be detected by the calculation. We therefore analyzed the effect of plant growth on the content of insoluble form of N in soil.

In Fig. 3, the contents of bio- and acid-soluble N in the planted soil were compared with those in the unplanted soil. The content of bio-soluble N in the planted soil was lower than that in the unplanted soil in any of the Fluvisol and Arenosol treatments, although the difference was statistically significant only in some of them. By contrast, the content of bio-soluble N in the planted soil was higher in most of the Andosol treatments. The content of acid-soluble N that is less soluble than bio-soluble N was lower in the planted soil than in the unplanted soil in any soil treatments.
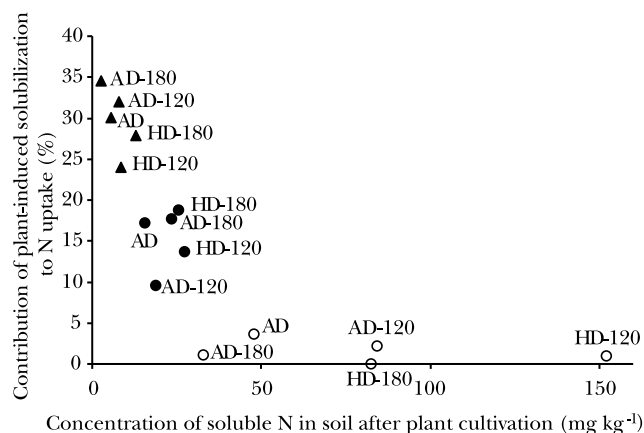

Fig. 2. Relationship between the contribution of plant-induced solubilization to N uptake and the content of soluble N remaining in the planted soils after the pot experiment. ○, Andosol treatment; ●, Fluvisol treatment; ▲, Arenosol treatment.
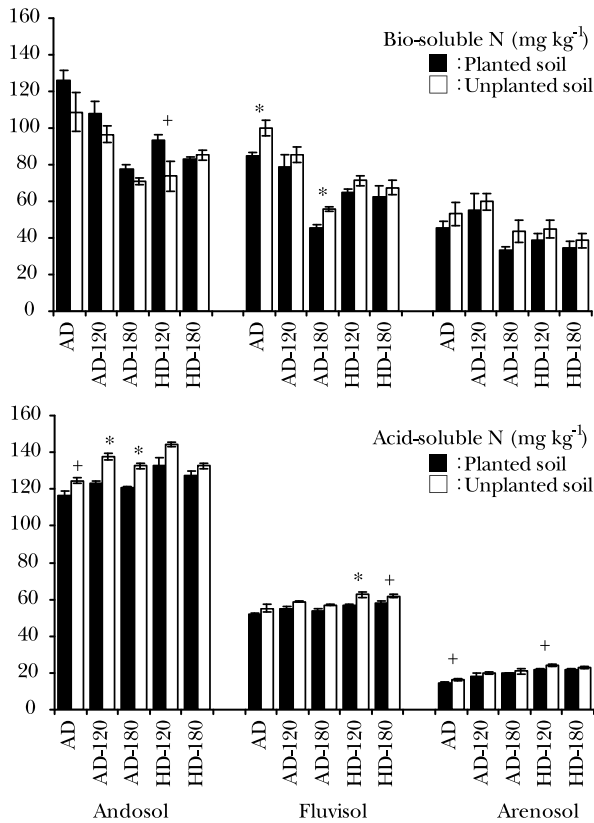
Fig. 3. Contents of bio-soluble and acid-soluble N in the planted and unplanted soils after the pot experiment. Bars denote means and standard errors of three replicates for planted soils and two replicates for unplanted soils. Bars with * and + indicate significant differences between the planted and unplanted treatments at the level of $P<0.05$ and $P<0.1$, respectively (t-test).

Discussion

Our results indicated that komatsuna grown in pots could cause solubilization of soil N significantly. For some of the Arenosol treatments, the amount of plant-induced solubilization exceeded 30% of the amount taken up by the plant (Table 2), and the percentage corresponded well with the degree of depletion of soluble N in soil (Fig. 2). This relationship suggests that the depletion of soluble N in soil below a certain threshold is necessary for significant contribution of plant-induced solubilization. In our study, the threshold value was around 30 mg kg^{-1}, but this value may change with the growth conditions and also by the soil sampling methods; i.e., the concentration of soluble N in the vicinity of roots could be much lower.

The results presented in Fig. 3 indicated the presence of plant-induced solubilization of bio- and acid-soluble N in the Fluvisol and Arenosol treatments, although many of the differences between the planted and unplanted soils were not statistically significant partly due to the limited number of replications. These two forms of insoluble N must have acted as the source of N solubilized by plants.

Since the concentration of dissolved organic N in the soil solution was higher than that of inorganic N only for the initial few weeks of the experiment when transpiration was negligible and it became too low to be determined accurately thereafter (data not shown), it is likely that the solubilized N had been mineralized before the plants absorbed it.

The results in Fig. 3 also revealed the presence of plant-induced solubilization of acid-soluble N in the Andosol treatments. Some of the acid-soluble N solubilized by plants remained in the soil as more labile bio-soluble N, due probably to the limitation of plant growth by P.

The high contribution of plant-induced solubilization to N uptake has also been reported at least for white lupin (Watanabe et al., 2006), sorghum (Okamoto et al., 2004) and *F. vivipara* (a grass growing in sub-arctic montane in north Sweden) (Jonasson et al., 2006). In these studies, both planted and unplanted soils were examined, by which the contribution of solubilization by the plant could be estimated in a way similar to ours. The effect of arbuscular mycorrhizal fungi seems to be negligible in all cases except for sorghum, because komatsuna and white lupin are known as non-mycorrhizal species (Usuki et al., 2007; Oba et al., 2001), and *F. vivipara* was non-mycorrhizal in the study area (Jonasson et al., 2006).

In the case of white lupin, the contribution was about 60% when it was grown in a volcanic ash soil to which air-dried cattle farmyard manure had been applied at the rate of about 0.5%. The amount of N supplied by plant-induced solubilization was larger than that of plant-induced depletion of 0.45 M H$_2$SO$_4$-extractable organic N, suggesting that acid-insoluble N was also released and utilized by the lupin. In the case of sorghum, the amount of N solubilized seems to have exceeded that of N taken up by plants, because the concentration of inorganic N in the planted soil was even higher than that in the unplanted control when it was grown in a mixture of soil and vermiculite to which rice bran with or without straw had been applied. Finally, solubilization of N by *F. vivipara* was more intensive than that by komatsuna, lupin and sorghum, because the amount of N uptake was several times greater than the amount of N mineralized in the unplanted control when it was grown on-site in a peat soil.

Since the contribution of the solubilization to N uptake by the plant can vary not only with the plant species but also with the growth condition especially the availability of soluble N, it is difficult to compare its contribution among the plant species. However, it seems that plant-induced solubilization was not caused by the limited availability of soluble or inorganic N. Plant-induced solubilization occurred in our Andosol treatments even though soluble N in the soil had not been fully depleted by komatsuna (Fig. 2). Sorghum and lupin solubilized and utilized N when inorganic N was abundant in soil (Okamoto and

Okada, 2004; Watanabe et al., 2006). The concentration of inorganic N in soil planted with *F. vivipara* became higher than that in the unplanted soil in half of the treatments (Jonasson et al., 2006). These observations are different from those reported by Weintraub and Schimel (2005), which suggested that solubilization of N by protease was stimulated by limited N availability to microbes and plants in Alaskan arctic tundra.

It is therefore unlikely that our results can be applied to other plants and ecosystems especially to natural plants growing under a limited supply of N. At least in komatsuna grown in fertilized soil, the depletion of soluble N in soil seems to be important for the increase in the contribution of the plant-induced solubilization to uptake but not for the occurrence of the solubilization in the root zone. In a broader sense, plant-induced N solubilization is not always a result of an active response of plants to absorb more N from soil.

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