STUDY ON THE GROWTH AND UTILIZATION OF HAIRY VETCH (*Vicia villosa* Roth) AS A GREEN MANURE IN A SUBTROPICAL REGION

(亜熱帯域における緑肥としてのヘアリーベッチの生育および利用に関する研究)

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A dissertation entitled STUDY ON THE GROWTH AND UTILIZATION OF HAIRY VETCH (*Vicia villosa* Roth) AS A GREEN MANURE IN A SUBTROPICAL REGION submitted to The United Graduate School of Agricultural Sciences, Kagoshima University, Japan by FAJRI ANUGROHO in partial fulfillment of the requirements for the degree of DOCTOR OF AGRICULTURE is hereby approved on the recommendation of

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Abstract

Phosphorus (P) is an essential for plant growth. Crop production is often limited by low P in acid soils of the subtropical agriculture. Several experiments have been conducted to assess an appropriate legume cover crop and green manure in subtropical soil conditions for the subsequent crop production in sustainable agriculture. Several winter legume plants were selected under pot condition with the P-deficient and P-sufficient soils in a short and long growing period. The growth of P-deficient tolerant plant was observed in fields for a long growing period.

In a short growing period, the biomass percentages of shoots and roots were 66 and 99 for hairy vetch, while 64 and 89 for crimson clover, relatively higher than those of pink clover, red clover and white clover, in comparison with the P-sufficient condition. In a long growing period, the biomass of shoot and root of hairy vetch were higher than that of clover species in February and March. Generally, the nutrient uptake of shoot and root of plants had a similar pattern to their biomass. Under pot conditions, hairy vetch is an appropriate legume in subtropics, regarding to the high biomass and nutrient uptake.

The biomass and nutrient uptake of hairy vetch grown under four subtropical soils showed a similar level. Hairy vetch sown within October-November and harvested February-April in subtropics was reached the shoot biomass averages of 4000 kg ha^{-1}, associated with nitrogen (N) accumulation of 140 kg ha^{-1}. Moreover, the shoot biomass of HV sown in October and December when harvested in February, March and April showed a significant difference. The 2 months delay in sowing date found the shoot biomass losses of 222 $x10^{-3}$kg m^{-2} in February, 129 $x10^{-3}$kg m^{-2} in March, 124 $x10^{-3}$kg m^{-2} in April, respectively.

Weeds, dominated by *Biden pillosa*, were effectively suppressed by the percentage of 71-87 through hairy vetch living mulch in the growing period of February-April, and by

the percentage of 37-65 at the desiccated stage in May, in comparison with the fallow soil. In contrast, the residue of hairy vetch stimulated the weed growth by 3-6 times during 63 and 84 days after burying; it may due to the richness of light and nutrients.

Hairy vetch was selected as an appropriate winter legume cover crop with higher contribution of organic matter, nutrient accumulation and weed suppression in the agriculture soils in a subtropical region. Hairy vetch is a better green manure legume among winter legume cover crops under P-deficient and P-sufficient condition either in short or long growing periods. In addition, crimson clover showed higher biomass tolerance in P-deficient condition in a short growing period, but lower biomass in a long growing period; in contrast with pink clover. Hairy vetch can grow under subtropical soil conditions within pH of 5.7-7.5, resulting in high biomass and nutrient accumulation. The nitrate-N content was increased during February to May. The release of nitrate-N content in no-tillage was slower than tillage system. In soil amended with hairy vetch residue, the corn growth showed higher in no-tillage than tillage. The living mulch of hairy vetch showed better weed suppression than the dead mulch of hairy vetch and fallow soils, but the weed growth was 3-6 times higher than fallow soils after incorporation. Therefore, the utilization of hairy vetch with either no-tillage or tillage systems in the agricultural practices should be integrated with the nutrient demand of the subsequent crop in subtropical regions.

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Chapter 1

General Introduction

Many people are deeply concerned about the future food supply of the world. A wider range of crops can be produced in the tropics and subtropics than elsewhere. Increasing supply means more agriculture development; including produce more crops by increasing the applications of fertilizer, tillage intensity, herbicide and pesticide into the soil (Hallberg, 1989; Hiltbrunner et al. 2007). In the short-term cultivation, those factors can stimulate crop production. However, the soil productivity gradually decreased and became a serious problem for a long cultivation period. The agriculture intensification has been markedly contributed to the land degradation causing the soil and water pollutions (Edmeades 2003).

Soil acidification is one of the most important land degradation issues facing subtropical agriculture. Harvest of high-yielding crops with low organic input in the soil play the significant role in increasing soil acidity (Bayer *et al*. 2001; Tiessen *et al*. 1994). Phosphorus (P) is one of the limiting nutrients in acidic soils which often have high P fixing capacity due to their high Al and Fe oxide concentrations. Most of the applied P through mineral fertilizers was gradually reacts with Fe and Al compounds in the acid soils and is transformed into relatively insoluble P compounds. Liao *et al.* (2006) noted that some legumes have a number of adaptive mechanisms for growing in deficient P soils, where roots may exude organic compounds to mobilize P from bound P pools in the soil.

On the other hand, the tillage intensity and high rainfall in Okinawa causes the soil prone to erosion. Erosion may transport the major nutrients out from the agriculture land, and left infertile soil. Similarly, both factors stimulated weed emergence and establishment throughout the year in Okinawa (Ishimine et al. 2004; Hossain 2005). The abundance of weeds may harbor insects, disease and nematode pests that are harmful for the main crop (Derr 2004). Herbicide application is a common method for weed control in cropping systems (Hiltbrunner et al. 2007). Those factors should be faced by an integration of cover crop and conservation tillage in a cropping system has been an alternative practice to attain long-term weed management (Blackshaw et al. 2006; Peigne et al. 2007).

A winter legume cover crop (WLCC) has been well integrated into a sustainable cropping system in temperate regions. Maximizing organic residue by WLCC in soil is a strategy for improving soil quality in agricultural practices (Bayer *et al*. 2000; Zanatta *et al*. 2007). Moreover, WLCC fix atmospheric N in their plant tissues in a symbiotic or mutually beneficial relationship with rhizobium bacteria. Another advantage of WLCC is weed suppression, but this often puts too high a competitive pressure on the main crop. However, the large variability among WLCC, including vetch and clover species, indicates that species selection is a very important aspect of the development of low input cropping systems (Den Hollander *et al.* 2007).

The utilization of several winter cover crops, with respect to the potential biomass for weed suppression and N accumulation for subsequent crop, has been observed (Kuo and Jellum 2002; Seo et al. 2006; Hiltbrunner et al. 2007). White clover is a highly productive winter cover crop in temperate regions, but less productive in subtropical Okinawa, Japan. Among clover species, white clover had clearly the lowest level of growth rate, dry matter, initial leaf area development and canopy height (Den Hollander et al. 2007a). Teasdale and Daughtry (1993) reported that living mulch with hairy vetch as a cover crop has a better ability to suppress weeds in comparison with plant residue mulch. However, incorporation of a cover crop into an intercropping system has been reported to result in severe competition with the main crop (Den Hollander et al. 2007b). Therefore, the utilization of cover crops must be evaluated before they will be introduced to a cropping system.

Hairy vetch, as a preferred winter legume cover crop in temperate regions to supply N and suppress weeds in cropping systems, has been well documented (Blackshaw et al. 2006; Peigne et al. 2007). Hairy vetch produces a natural level of biomass approximately 3000 to 5000 kg ha^{-1}, with associated N content ranging from 100 to 150 kg ha^{-1} (Teasdale and Mohler 2000). The reduction percentages of weed density and weed biomass were 70-78 and 52-70, respectively compared with fallow treatment (Teasdale and Daughtry 1993). Similarly, Teasdale and Mohler (2000) reported that the inhibition percentage of weed emergence was greater than 75, is consistently achieved only when mulch biomass and mulch thickness of hairy vetch exceeds 8000 kg ha^{-1} and 0.1 m, respectively. Moreover, the weed suppression may due to the cyanamide, methanol and ethyl acetate contained in hairy vetch residue, together with competition for light, nutrients and water (Kamo et al. 2003; Hill et al. 2007).

A better supply of N is always needed for sustainable plant production. Biological N_2 fixation, especially the symbiotic association between legumes and rhizobia, can provide substantial amounts of N to plants and soil, which reduces the need for industrial fertilizers iIn temperate region (McVay et al. 1989; Clark et al. 1995; Kuo and Jellum 2002).

Conservation tillage included no-tillage has many advantages in crop production system (Peigne et al. 2007), and weed control management (Hiltbrunner et al. 2007). In contrast, conventional tillage may cause tons of nutrients are dissolved and transported with runoff sediments from agricultural land annually (Oyedele and Aina 1998). High decomposition rates of soil organic matter associated with degradation processes which is accelerated by tillage operation in cultivated soils present a very disturbing problem. Sanchez and Logan (1992) showed that soil organic matter in the tropics decomposes five times faster than in temperate regions. N supply by shoot and root of hairy vetch residue in corn production through tillage and no-tillage systems may increase inorganic-N level in soils and contribute to better agronomic practices for sustainable agriculture.

In the present study, several experiments have been conducted to determine: (i) selection of the winter legume cover crops regarding to their biomass and nutrient uptake, (ii) growth and development of hairy vetch as influenced by sowing and harvesting date, (iii) effect of sowing and harvesting date on the growth and nutrient uptake of hairy vetch and soil chemical properties, (iv) Effect of cover cropping and subsequent incorporation of hairy vetch on weed suppression and soil inorganic-N, (v) Soil inorganic-N distribution and corn growth influenced by tillage and no tillage of hairy vetch.

Chapter 2

Productivity of Hairy Vetch as a Winter Leguminous Cover Crop

2.1. Growth, Nitrogen Fixation and Nutrient Uptake of Winter Legume Cover Crops in Subtropical Soil Conditions: Plant Selection

2.1.1. Introduction

Soil acidification is one of the most serious forms of land degradation in subtropical agriculture. Soils that are acidic or are acidifying generally exist where rainfall exceeds 450 mm year^{-1} (Scott *et al.,* 2000), potentially limiting crop production on the most productive agricultural land. The harvesting of high-yielding crops also depletes organic matter from agricultural fields, a process that both contributes to and is accelerated by soil erosion when soil is left bare after crop harvests (Bayer *et al*. 2001; Oyedele and Aina 1998; Sanchez and Logan 1992; Tiessen *et al*. 1994).

Phosphorus is often a limiting nutrient in acidic soils owing to the high P-fixing capacity of these soils. Most of the P in mineral fertilizers reacts with Fe and Al compounds and is transformed into relatively insoluble forms. The use of plants tolerant to low P availability is employed to maintain plant productivity on acid soils. Some legumes can exude organic compounds that mobilize P from bound P pools in the soil (Liao *et al.* 2006).

Winter legume cover crops are an important biological tool for sustainable agriculture as they can increase inputs of organic matter to the soil, leading to improved soil aggregate stability and therefore reduced runoff and erosion. Maximizing organic residues through the use of winter legume cover crops is an important strategy for improving soil quality in agricultural practices (Bayer *et al*. 2000; Zanatta *et al*. 2007).

Moreover, winter legume cover crops fix atmospheric N and suppress weeds. However, the large variability in productivity among winter legume cover crops, which include species of vetch and clover, means that species selection is an important consideration in developing low-input cropping systems (Den Hollander *et al.* 2007).

The effect of hairy vetch on maize productivity in the relay cropping system in subtropical regions was investigated by Zougmore *et al.* (2006). However, the performance of hairy vetch as a cover crop compared with that of other vetch and clover species under subtropical conditions is not well understood.

The objectives of the present study were to: (i) select a species of vetch or clover that is tolerant of P deficiency over a short growing period, (ii) confirm the growth of hairy vetch and four clover species during a long growing period when P is not limiting, and (iii) examine the field performance of hairy vetch in four different soils in subtropical Okinawa by determining biomass and nutrient uptake.

2.1.2. Materials and Methods

Pot trials with clover and vetch species

We conducted two sets of experiments under controlled conditions to evaluate the performance of four species of clover and four species of vetch. The species examined were perennial white clover (*Trifolium repens* L.), biennial red clover (*Trifolium pretense* L.), annual crimson clover (*Trifolium incarnatum* L.), annual pink clover (*Trifolium gracilentum* L.), annual hairy vetch (*Vicia villosa* Roth), annual tiny vetch (*Vicia hirsuta* L.), annual narrowleaf vetch (*Vicia angustifolia* L.), and annual Chinese milk vetch (*Astragalus sinicus* L.).

The first experiment examined all eight species under two levels of phosphorus, P limiting (P-deficient) and P nonlimiting (P-sufficient) conditions. The second experiment examined the best performing vetch (hairy vetch) from the first experiment against the four clover species with three harvests of above-ground biomass over an extended growing season with P-deficient condition. The three harvests represented vegetative, flowering, and mature growth stages. The experiments were conducted at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan (26° N, 127° E). The plants were grown in 1/5000-a Wagner pots filled with 2.2 kg Akadama soil. Individual treatments were replicated with three pots in both experiments. The chemical properties of the soil in each experiment are shown in Table 2.1.1. In both experiments, N and K_2O requirements of plants were supplied by the addition of 0.5 g pot^{-1} of each as urea and K_2SO_4, respectively.

Experiment 1. Clover and vetch species under P-deficient and P-sufficient conditions

P-deficient and P-sufficient conditions were prepared by adding superphosphate to pots to achieve P_2O_5 absorption coefficient values of 0.01 and 0.10, respectively (Kitou et al. 2009). Ten seeds of each of the four clovers were sown on 19 October 2005. In a separate set of pots, seven seeds of each of the vetch species were sown on 2 November 2005. Clover and vetch species, as shown in Picture 2.1.1, were harvested on 14 December and 28 December 2005, respectively. Each treatment was performed with three replications.

Experiment 2. Selection of hairy vetch and clover species under P-sufficient conditions

We previously compared the growth of narrowleaf vetch, tiny vetch, and hairy vetch over a longer growing period (3-5 months after sowing) with P-deficient condition (Anugroho and Kitou 2007). In the present study, we repeated this experiment with hairy vetch and the four clover species. P_2O_5 was supplied at the same rate as in the P-sufficient condition of Experiment 1. Seeds were sown at a rate of 50 mg pot^{-1} on 6 November 2006. All plants were harvested on 6 February, 6 March, and 6 April 2007, as shown in Picture 2.1.2. Each plant was performed with three replications.

Experiment 3. Field performance of hairy vetch on four different soils

Field experiments were carried out at the Tropical Agriculture Research Front of the Japan International Research Center for Agricultural Science, Ishigaki, Okinawa, Japan (24° N, 129° E). Four different soils (yellow, red, dark red, and gray soil) in plots of 2 m×2.4 m at 2 m depth were used (Picture 2.1.3). The chemical properties of the four soils are shown in Table 2.1.1. Hairy vetch was sown on 24 November 2006, with four seeds per hill and 16 hills m^{-2}. No fertilizer was applied to the soils. Hairy vetch was harvested on 24 April 2007, as shown in Picture 2.1.4, by sampling all above-ground biomass within 0.5 m×0.5 m randomly placed quadrats per plot. Each treatment was performed with three replications.

Plant and soil analysis

Nitrogen-fixing activity was measured by the acetylene reduction assay method (Yoshida 1982) using gas chromatography (GS-2; Sensortec, Tokyo, Japan). Shoots and roots were dried at 70 °C for 72 h, weighed, and ground to a powder. Total C and N of plant samples were determined using a CHN Corder (JM10; G-Science Laboratory Company, Tokyo, Japan). Total P of plant material was determined by calorimetry after combustion at 450 °C for 1 h, and the resulting ash was dissolved in 1:30 (v v^{-1}) diluted nitric acid (Hafner *et al*. 1993). K, Ca, and Mg concentrations were determined by an atomic absorption spectrophotometer (Solaar 969; Japan Thermo Corporation, Tokyo, Japan) after Kjeldahl digestion with H_2SO_4 and H_2O_2.

Soils were air dried and passed through a 2-mm sieve. Soil pH and EC were measured in 1:2.5 and 1:5 (w v^{-1}) soil:water slurries, respectively. Total C and N of soils were determined using a CHN Corder (JM10; G-Science Laboratory Company, Tokyo, Japan). Ammonium-N and nitrate-N contents were determined by steam distillation after extraction with 2 mol L^{-1} KCl (Mulvaney 1996). The phosphate absorption coefficient was determined calorimetrically after absorption with 25 g L^{-1} NH_4HSO_4. Ca-bound phosphate was determined by extraction with 25 mL L^{-1} acetic acid. Exchangeable cations were determined by the Solaar 969 atomic absorption spectrophotometer after extraction with 1 mol L^{-1} ammonium acetate.

Statistical analysis

Data are presented as the means of three replicates with standard error of the mean of sets of values being statistically compared. Biomass and nutrient uptake data were analyzed by one-way analysis of variance (ANOVA) with a complete randomized block design and Fisher's LSD and Duncan's multiple range tests at $P<0.05$ for post hoc comparison of means of significant ANOVA results. Statistical procedures were performed with the software package SPSS 14.0 for Windows (SPSS, Chicago, IL, USA).

2.1.3. Results

Biomass, N fixing activity, and shoot P content of plants under P-deficient and P-sufficient conditions

Under P-sufficient conditions, crimson clover and pink clover produced significantly greater shoot biomass than white clover and red clover (Fig. 2.1.1A), and crimson clover, red clover, and pink clover produced significantly greater root biomass than white clover (Fig. 2.1.1B). Under P-deficient conditions, the shoot and root biomasses of crimson clover were significantly greater than those of the other clover species. The shoot and root biomasses of hairy vetch were significantly greater than those of the other vetch species under both P-deficient and P-sufficient conditions. The biomass percentages of shoots and roots of crimson clover were 64 and 89, respectively, of those under P-sufficient condition; these were higher than the corresponding percentages of the other clover species. Similarly, the biomass percentages of shoot and root of hairy vetch under P-deficient conditions were 66 and 99, respectively, of those under P-sufficient conditions, and these were higher than those of the other vetch species. These results show that crimson clover and hairy vetch were tolerant of P-deficient conditions.

Nitrogen-fixing activity under P-sufficient conditions was significantly higher in white clover than in the other clover species; however, there were no significant differences between clover species under P-deficient conditions (Fig. 2.1.1C). The N-fixing activities of hairy vetch and narrowleaf vetch were significantly higher than that of tiny vetch under both P-sufficient and P-deficient conditions. The percentages of N-fixing activities of crimson clover and hairy vetch under P-deficient conditions were 68 and 83 of the respective N-fixing activities under P-sufficient conditions. Narrowleaf vetch and tiny vetch recorded the percentages of N-fixing activities under P-deficient conditions that were 88 and 181, respectively, of what they produced under P-sufficient conditions; however, the growth of both plants was less than that of hairy vetch. Nitrogen-fixing activity was not detected in Chinese milk vetch. Shoot P contents were not significantly different among clover species under both P-sufficient and P-deficient conditions (Fig. 2.1.1D). The shoot P content of narrowleaf vetch was significantly higher than that of hairy vetch and tiny vetch under P-sufficient conditions, but there were no significant differences between other vetch species. Clover and vetch species had similar shoot P contents under P-deficient conditions, and these percentages were 48-63 of the comparative P contents under P-sufficient conditions.

Biomass, N-fixing activity, and nutrient uptake of plants under P-sufficient conditions over a longer growing period

The biomass of shoots and roots of plants increased with each successive harvest, that is, in February, March, and April (Fig. 2.1.2). Hairy vetch produced greater shoot biomass and root biomass than the clover species at all harvests, except that shoot biomass of pink clover and root biomass of red clover and pink clover were greater (but not significantly so) than those of hairy vetch (Fig. 2.1.2A, B). Moreover, the shoot biomass of pink clover was significantly greater than that of other clover species harvested in March and April. The N-fixing activity of hairy vetch was significantly greater than that of the clover species in February and March, except for white clover in March (Fig. 2.1.2C); in April, however, hairy vetch recorded the lowest N-fixing activity. In February, the N-fixing activity of hairy vetch was 61 μmol h^{-1} pot^{-1}, double the levels of the clover species.

Nitrogen uptake in shoots and roots in all plants followed similar increases to those observed for biomass, except that shoot N uptake did not increase in the April harvest in hairy vetch and crimson clover (Fig. 2.1.3A). Hairy vetch harvested in February and March had significantly greater shoot N uptake than the clover species. The roots of red clover harvested in April had the highest N uptake of all plants. The C/N ratios varied in the ranges of 11.7-20.7 in the shoots and 13.0-17.1 in the roots of clover species; the corresponding ranges for hairy vetch were 9.1-14.5 and 10.3-13.0 (Fig. 2.1.3B). The C/N ratios of shoots and roots of hairy vetch were generally significantly lower than those of clover species in February, March, and April, but the shoot C/N ratio of hairy vetch in April was similar to that of white clover and red clover.

Shoot P uptake also tended to follow shoot biomass, although in hairy vetch P uptake did not increase from the March to April harvests (Fig. 2.1.4A). Root P uptake increased between February and March, except in crimson clover, and increased again in April, except in hairy vetch and pink clover. Meanwhile, red clover displayed a clear increase of P uptake in the shoots and roots between March and April. Generally, P uptake in the shoots and roots of hairy vetch was relatively greater than in clover species in February and March. In addition, shoot mineral uptake of plants increased during February, March, and April (Fig. 2.1.4B-D), except for shoot K uptake in hairy vetch and pink clover and shoot Mg uptake in crimson clover in April. Red clover displayed significantly greater root uptake of P and minerals in April than the other plants. Shoot K uptake of hairy vetch harvested in February was significantly greater than those of the four clovers. Root K uptake of red clover in February was significantly greater than those of the other four species. Shoot Ca uptake of hairy vetch and pink clover was significantly greater than those of white clover, crimson clover, and red clover in February and March, and in the case of pink clover also in April, when hairy vetch was not significantly different than any other species. Root Ca uptake of hairy vetch harvested in March was significantly greater than those of white clover and crimson clover. Shoot Mg uptake of hairy vetch and white clover harvested in February was significantly higher than that of pink clover, whereas root Mg uptake of hairy vetch in February and March was significantly higher than that of the clover species, except for red clover, in February. In general, there were very few significant differences between species in shoot uptake of P or minerals at the April harvest.

Biomass and nutrient uptake of hairy vetch in field trials on four different soils

The shoot biomass and nutrient uptake of hairy vetch were not significantly different between the four different soils (Fig. 2.1.5, Table 2.1.2). Shoot biomass was in the range 400-500 g m^{-2}. Nitrogen uptake by shoots was in the range of 11.5-15.4 g m^{-2} and P, K, Ca, and Mg uptake by shoots displayed ranges of 0.9-1.7, 5.7-8.4, 3.4-6.4, and 0.7-1.2 g m^{-2}, respectively. The P concentrations of hairy vetch grown in the more neutral pH dark red and gray soils were significantly higher than those grown in the more acidic yellow and red soils. The Ca concentration of hairy vetch grown in the neutral gray soil was significantly higher than that in the acid soils. C/N ratios ranged from 12.8 to 14.4.

2.1.4. Discussion

With a short growing period under P-deficient conditions, shoot and root biomass (Fig. 2.1.1) and root P content (data not shown) of hairy vetch and crimson clover were greater than those of other vetch species and other clover species, respectively. The percentages of shoot biomasses of narrowleaf vetch, tiny vetch, and Chinese milk vetch with P sufficient and a short growing period were 44, 26, and 16, respectively, of that of hairy vetch. These results are in line with those of an earlier study in which the percentages of the shoot biomass of narrowleaf vetch and tiny vetch grown under P-sufficient conditions over a long growing period in a subtropical region ranged from 42-62 and 12-25, respectively, of the value of hairy vetch (Anugroho and Kitou 2007). Even though Chinese milk vetch is widely used as a cover fertilizer crop in rice fields in temperate regions (Samarajeewa *et al*. 2005), it did not grow well under the subtropical conditions of the present study.

In the present study, over a longer growing period and with P sufficient, the total biomass of shoots and roots of hairy vetch in the vegetative (February) and flowering (March) stages was almost double that of clover species excluded pink clover in the flowering stage; similar results were found between hairy vetch and white clover in a northern temperate region (Brandsaeter *et al*. 2008). The greater biomass of hairy vetch may be due to its high N-fixing activity in the late vegetative and early flowering stages (Anugroho *et al*. 2009a). Unlike hairy vetch and pink clover, the flowering stage of white clover and crimson clover was occurred in April, whereas red clover was still in the vegetative stage during the experimental period.

In the field experiments, the pH of the soils ranged from 5.7 to 7.5, which is within the range of 4.9 to 8.2 recommended for hairy vetch in temperate regions (Duke 1981). The shoot biomass production of hairy vetch in the four different soils was similar to previous results reported from subtropical and northern temperate regions (Anugroho *et al*. 2009b; Brandsaeter *et al*. 2008). Incorporation of hairy vetch into soil reduced N fertilizer demand to 90 kg ha^{-1} for tomato production in a northern temperate region (Sainju *et al.* 2002).

Even though the shoot biomass of hairy vetch increased markedly from February to March and increased further in April, the N-fixing activity and N uptake decreased. The decreases probably occurred due to the transition from vegetative growth to flowering in March. The percentage of the shoot N concentrations in April (maturity stage) declined to 58 of those measured in March (data not shown). In addition, the shoot C/N ratios of hairy vetch, white clover, and red clover were lower than those of crimson clover and pink clover; represented as the potential mineralization of those plant residues. Brandsaeter *et al*. (2008) reported that mineralization rates of N derived from hairy vetch and white clover organic residues were higher those from crimson clover, which would be consistent with the former two having lower C/N ratios than crimson clover. Seneviratne (2000) reported that rapid N mineralization generally occurred in plant residues with lower C/N ratios and lower lignin and polyphenol contents.

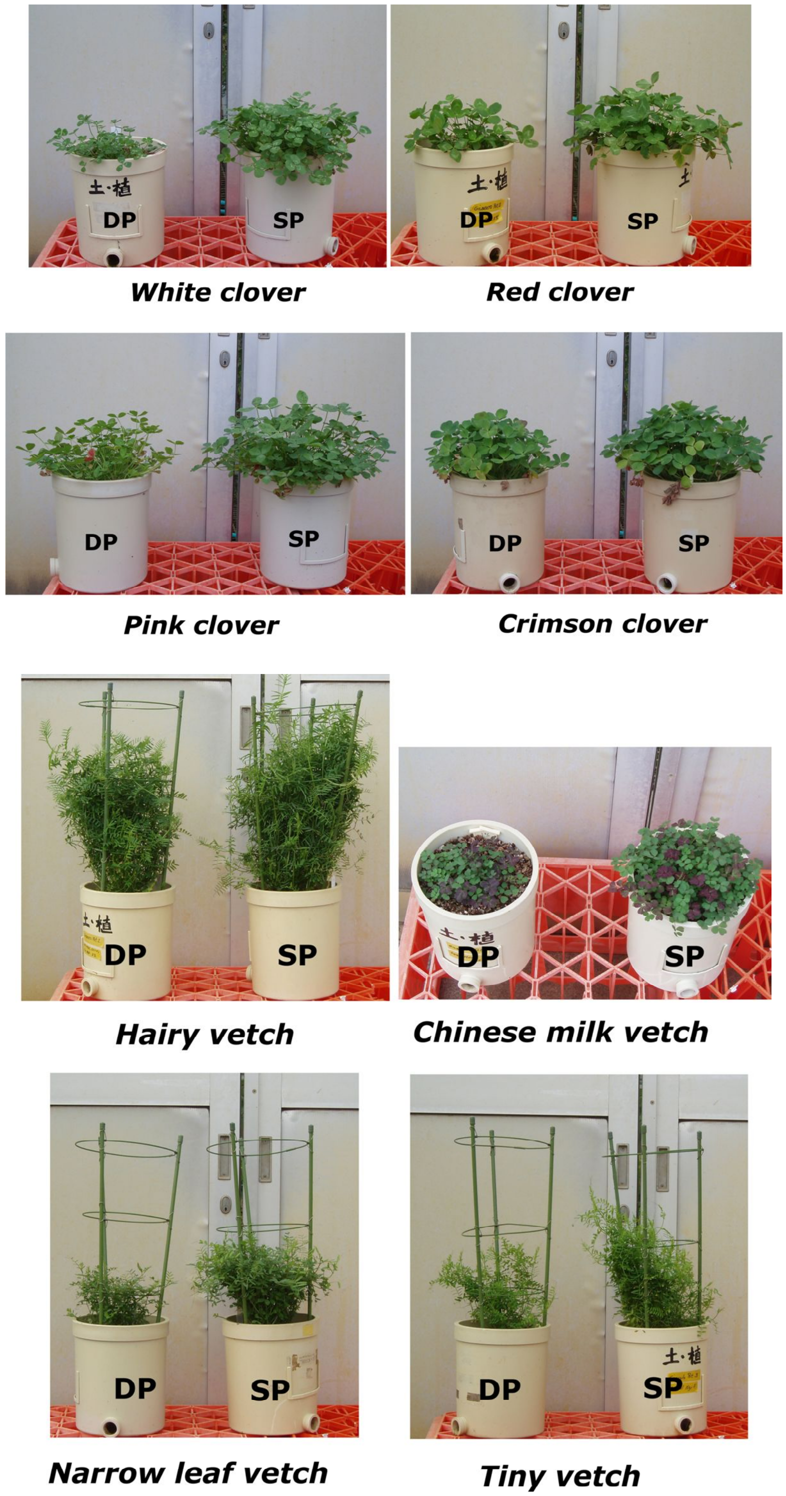
In the pot experiments, the decrease in shoot P uptake of hairy vetch in April, even though biomass increased, may have been due to the number of yellow leaves increased, senesced and were shed in April (Anugroho *et al*. 2009a), and hairy vetch displayed significantly higher shoot K uptake than clover species during the vegetative growth stage (February) but no other stage. Shoot K uptake decreased after the flowering stage, as also reported from studies in temperate regions (Caballero *et al*. 1996; Sainju *et al*. 1998) and subtropical regions (Anugroho *et al*. 2009a). Generally, P and K uptake of roots tended to be stable over time, may have been due to a decline in P and K concentrations from the early flowering stage. The decrease in P and K concentrations may have been related to the decrease in N-fixing activity as reported by (Hogh-Jensen 2003; Olivera et al. 2004).

This study suggests that hairy vetch is a more appropriate winter legume cover crop than clover species in subtropical regions under either P-deficient or P-sufficient soil conditions. We confirmed that hairy vetch can be grown as a cover crop in four different soils with no added fertilizer. On acid soils in subtropical regions, low inputs of chemical fertilizer and high inputs of organic matter derived from hairy vetch may be a more sustainable agricultural system than systems based on high chemical fertilizer inputs and clover cover crops. Because of its low C/N ratio, hairy vetch may be a suitable green manure for vegetable crops with high N requirements. Further study should focus on the distribution of inorganic N in subtropical soils produced by incorporating or mulching with hairy vetch. This will better determine the suitability of hairy vetch as a low-cost green manure for meeting the N demand of subsequent high-yielding vegetable crops.

Table 2.1.1. Initial chemical properties of different soils used in three different experiments

	Experiment 1 (Akadama soil)	Experiment 2 (Akadama soil)	Experiment 3			
			Yellow soil	Red soil	Dark red soil	Gray soil
pH	5.8 ± 0.0	6.7 ± 0.0	5.9 ± 0.0	5.7 ± 0.0	6.4 ± 0.1	7.5 ± 0.1
EC ($mS\ m^{-1}$)	12.3 ± 0.4	34.5 ± 0.8	2.5 ± 0.1	3.8 ± 0.2	3.0 ± 0.0	10.7 ± 0.1
Total C ($g\ kg^{-1}$)	13.6 ± 0.4	14.4 ± 0.1	3.2 ± 0.1	7.0 ± 0.1	8.2 ± 0.2	28.9 ± 0.2
Total N ($g\ kg^{-1}$)	1.1 ± 0.1	1.2 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	1.0 ± 0.1	0.6 ± 0.0
NH_4^+ ($mg\ kg^{-1}$)	2.7 ± 0.5	5.0 ± 1.2	3.2 ± 1.2	7.0 ± 0.7	5.0 ± 1.2	8.0 ± 1.7
NO_3^- ($mg\ kg^{-1}$)	12.8 ± 0.7	6.7 ± 0.0	1.2 ± 0.5	3.1 ± 1.7	0.9 ± 0.0	3.5 ± 0.7
P_2O_5 Abs. Coef.† ($g\ kg^{-1}$)	21.1 ± 0.0	21.2 ± 0.2	3.3 ± 0.1	5.8 ± 0.5	8.2 ± 0.1	8.9 ± 0.2
Available P_2O_5¶	nd	nd	3.1 ± 0.0	1.1 ± 0.0	34.0 ± 3.0	499.7 ± 5.2
Ex.‡ K^+ ($mmol_c\ kg^{-1}$)	9.3 ± 1.3	97.5 ± 0.9	6.6 ± 1.2	2.6 ± 0.7	7.4 ± 1.3	6.8 ± 0.4
Ex. Ca^{2+} ($mmol_c\ kg^{-1}$)	48.4 ± 4.5	14.2 ± 1.4	21.6 ± 3.9	45.6 ± 3.5	146.1 ± 7.4	303.7 ± 8.6
Ex. Mg^{2+} ($mmol_c\ kg^{-1}$)	7.0 ± 1.3	6.2 ± 0.7	8.3 ± 0.3	13.9 ± 0.7	17.1 ± 1.2	10.4 ± 0.4

†) phosphate absorption coefficient; ‡) exchangeable cation; ¶) Ca bound phosphate; nd: no detected. Values are means ± standard deviation (n=3)



Picture 2.1.1. Performance of clover and vetch species under P-deficient (DP) and P-sufficient (SP) at 6 weeks after sowing in Experiment 1.

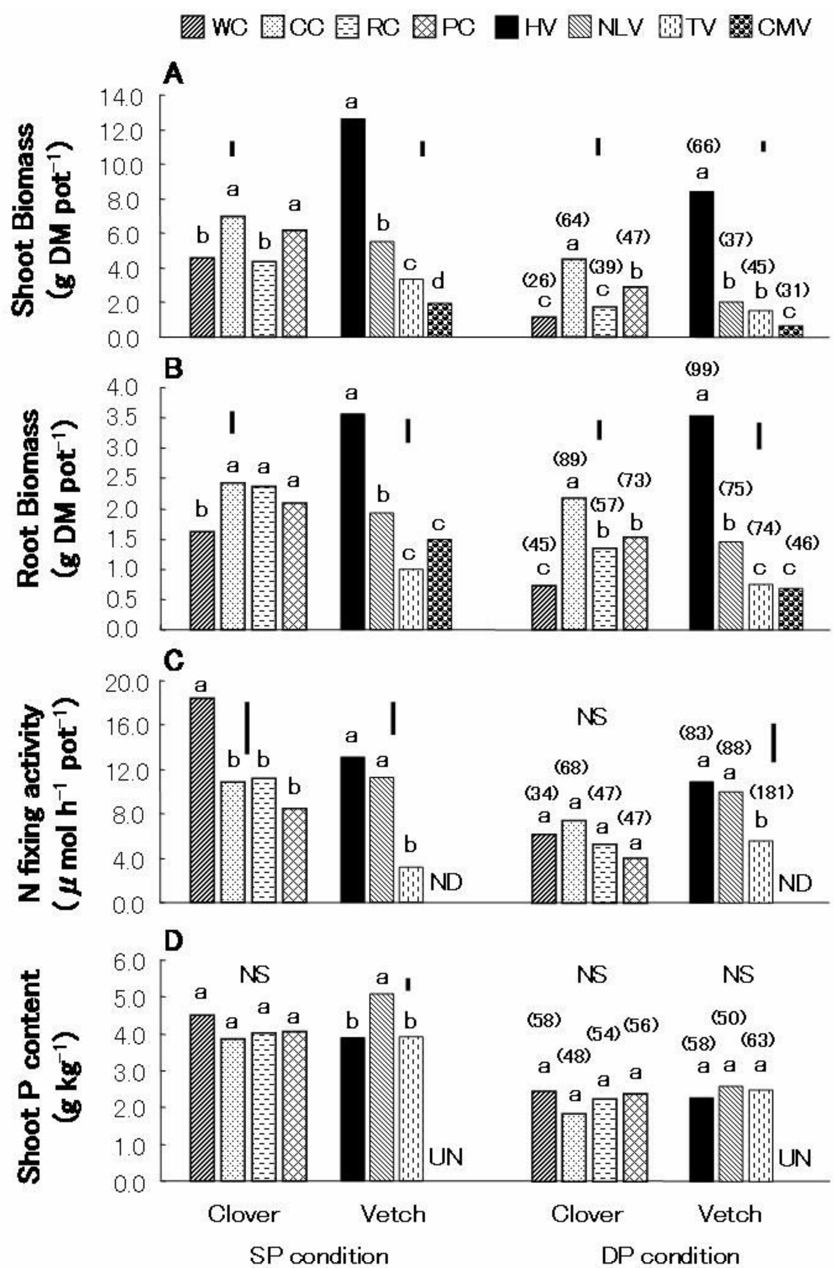


Figure 2.1.1. Biomass dry matter, N fixing activity and shoot P content of clover and vetch species grown at two different P levels (DP, deficient P and SP, sufficient P) in Experiment 1. WC, white clover; CC, crimson clover; RC, red clover; PC, pink clover; HV, hairy vetch; NLV, narrow leaf vetch; TV, tiny vetch; CMV, Chinese milk vetch; nd, no detected; un, unmeasured. Numbers in parentheses indicate the percentages of DP to SP ratios. Vertical bars show the Least Significant Difference (LSD) at $P<0.05$ (n=3). The different letters show a significant difference. NS: no significant difference.

Picture 2.1.2. Performance of hairy vetch and four clover species under P-sufficient (SP) in three different harvesting dates in Experiment 2.

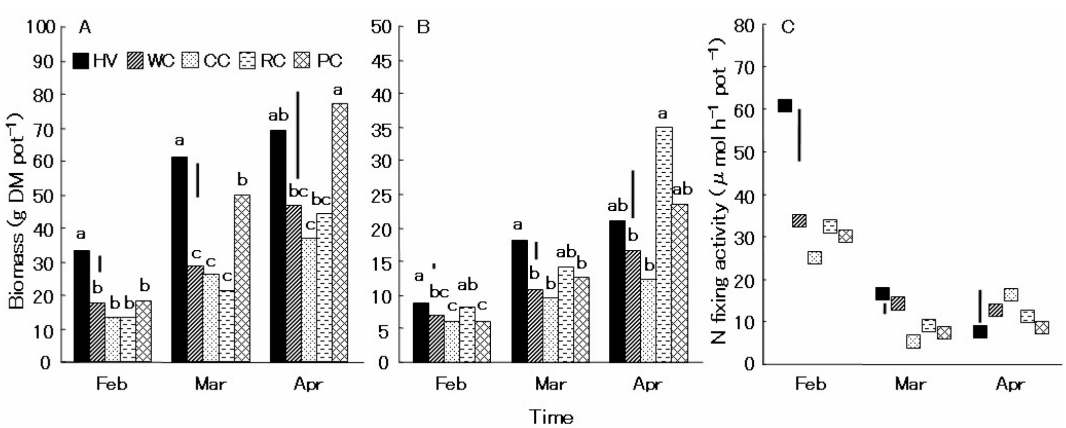


Figure 2.1.2. Shoot biomass (A), root biomass (B) and N fixing activity (C) of hairy vetch and clover species harvested from February to April in Experiment 2. HV, hairy vetch; WC, white clover; CC, crimson clover; RC, red clover; PC, pink clover. Vertical bars are significantly different according to LSD test (n = 3); columns with the same letter are not significantly different (NS) at $p < 0.05$.

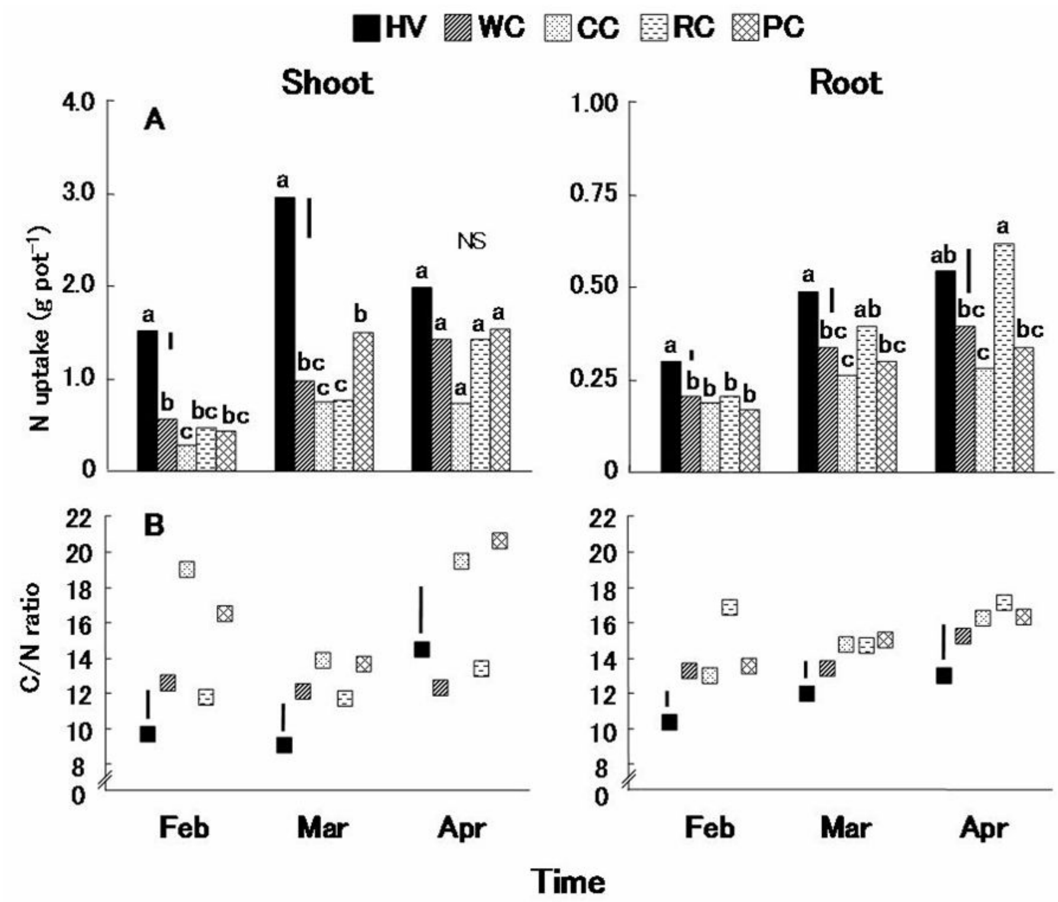


Figure 2.1.3. Fixed C, N uptake and CN ratios of shoot and root parts of hairy vetch and clover species harvested from February to April in Experiment 2. HV, hairy vetch; WC, white clover; CC, crimson clover; RC, red clover; PC, pink clover. Vertical bars represent significantly different according to LSD test (n = 3); columns with the same letter are not significantly different (NS) at $p < 0.05$.

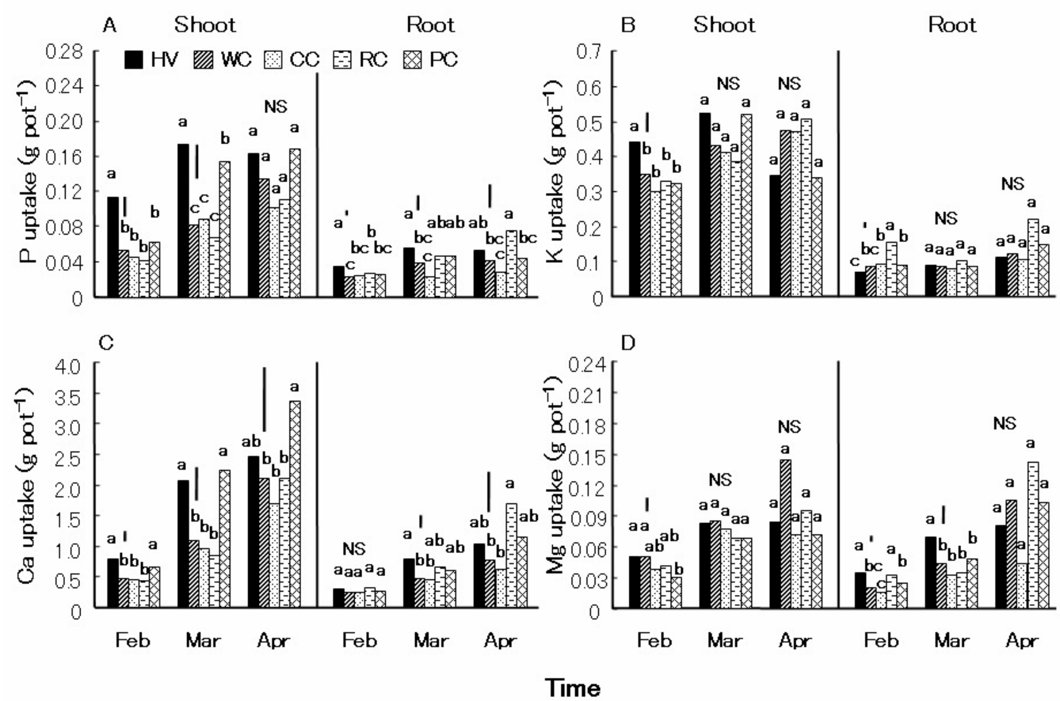
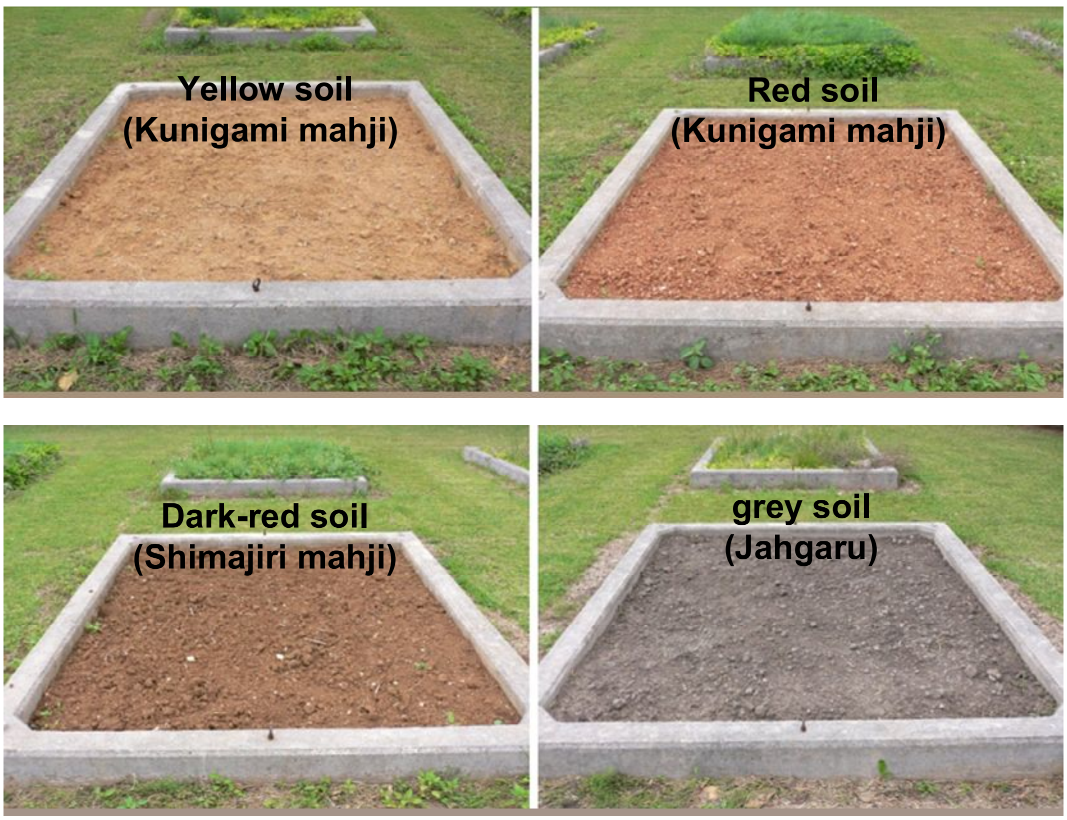


Figure 2.1.4. Phosphorus and mineral uptakes by shoot and root parts of hairy vetch and clover species harvested from February to April in Experiment 2. HV, hairy vetch; WC, white clover; CC, crimson clover; RC, red clover; PC, pink clover. Vertical bars represent significantly different according to LSD test (n = 3); columns with the same letter are not significantly different (NS) at $p < 0.05$.



Picture 2.1.3. Four different subtropical soils used in Experiment 3.

"Kunigami mahji"
Yellow soil

"Kunigami mahji"
Red soil

"Shimajiri mahji"
Dark-red soil

"Jahgaru"
Gray soil

Picture 2.1.4. Performance of hairy vetch on four different subtropical soils in April 2007.

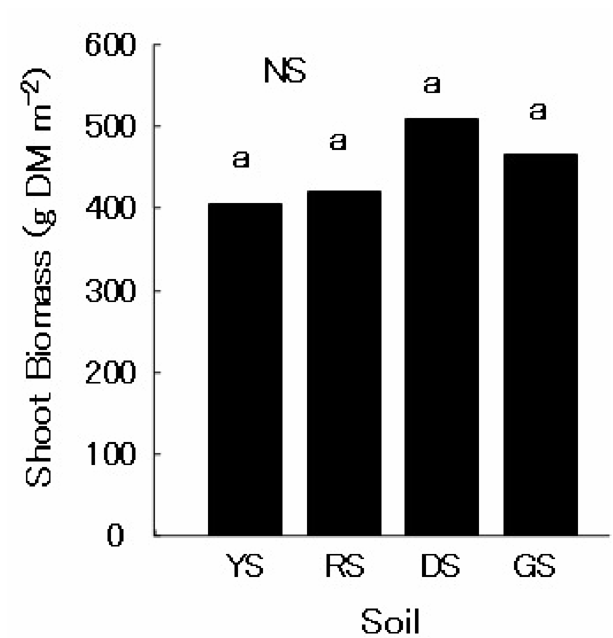


Figure 2.1.5. Shoot biomass of hairy vetch grown under four different soils in Experiment 3. YS, yellow soil; RS, red soil; DS, dark red soil; GS: gray soil. Columns with the same letter are not significantly different (NS) at $p < 0.05$.

Table 2.1.2. Nutrient concentration and uptake of hairy vetch grown under four different soils in Experiment 3

	N		P		K		Ca		Mg	
Concentration (g kg^{-1})										
Yellow soil (YS)	28.5	a	2.1	b	14.4	a	8.4	b	1.8	a
Red soil (RS)	31.9	a	2.1	b	17.4	a	10.4	b	1.9	a
Dark red soil (DS)	30.2	a	3.3	a	14.8	a	9.8	b	2.4	a
Grey soil (GS)	30.8	a	3.3	a	18.2	a	13.6	a	2.3	a
LSD (p<0.05)	NS		0.6		NS		2.8		NS	
Accumulation (g m^{-2})										
Yellow soil (YS)	11.5	a	0.9	a	5.7	a	3.4	a	0.7	a
Red soil (RS)	13.3	a	0.9	a	7.4	a	4.4	a	0.8	a
Dark red soil (DS)	15.4	a	1.7	a	7.5	a	4.9	a	1.2	a
Grey soil (GS)	14.3	a	1.5	a	8.4	a	6.4	a	1.1	a
LSD (p<0.05)	NS		NS		NS		NS		NS	

Within a column, means with different letters are significantly different using Fisher's least square difference (LSD) test at $P < 0.05$ ($n = 3$); NS, no significant difference.

2.2. Growth, Nitrogen Fixation, and Nutrient Uptake of Hairy Vetch Cultivars as Influenced by Sowing and Harvesting Date

2.2.1. Introduction

In subtropical Okinawa, weeds occupied agricultural fields throughout the year (Hossain 2005). *Amaranthus spinosus* L., *A. viridis* L., *Biden pilosa* L., *Chenopodium album* L., *Cyperus rotundus* L., *Digitaria ciliaris* (Retz.) Koeler, *D. timorensis* (Kunth) Balansa, *Oxalis corymbosa* DC., *Rottboellia exaltata* L., *Solanum nigrum* L., *Sonchus asper* (L.) Hill and *S. oleraceus* L. have been reported as major weed species in the winter and the spring seasons in subtropical Okinawa, Japan (Ishimine et al. 2004; Hossain 2005). The weeds were abundant in the left bare fields after harvesting of the summer crops, such as sweet potatoes, turmeric and corn, related to enough resources and no competition. The abundance of weeds may harbor insects, disease and nematode pests that are harmful for the main crop (Derr 2004). In recent years, tillage operations and herbicide applications have been two common methods of weed control in cropping systems (Hiltbrunner et al. 2007). However, when left bare after tillage operations, the soil is prone to erosion and leaching of herbicides into the surface and ground water (Hallberg 1989). The integration of a cover crop and conservation tillage in a cropping system has been an alternative practice to attain long-term weed management (Blackshaw et al. 2006; Peigne et al. 2007).

The use of several winter cover crops, with respect to the potential biomass for weed suppression and nitrogen (N) accumulation for the subsequent crop, has been observed (Kuo and Jellum 2002; Seo et al. 2006; Hiltbrunner et al. 2007). White clover is a highly productive winter cover crop in temperate regions, but is less productive in subtropical Okinawa, Japan. Among clover species, white clover had clearly the lowest level of growth rate, dry matter, initial leaf area development and canopy height (Den Hollander et al. 2007a). Most of the soils in Okinawa are characterized as acid soils. In our previous study, hairy vetch had the highest total biomass and N-fixing activity under a phosphorus (P)-deficient soil condition, followed by crimson clover, pink clover, red clover and white clover. Similarly, hairy vetch obtained a higher biomass, N-fixing activity and N uptake than that of four clover plants under favorable condition (Anugroho et al. 2007). Teasdale and Daughtry (1993) reported that living mulch, with hairy vetch as a cover crop, had a better ability to suppress weeds in comparison with plant residue mulch. However, the incorporation of a cover crop into an intercropping system has been reported to result in severe competition with the main crop (Den Hollander et al. 2007b). Therefore, use of cover crops must be evaluated before being introduced to a cropping system.

Hairy vetch, as a preferred winter legume cover crop in temperate regions to suppress weeds and supply N in cropping systems, has been well documented. The percentage of weed suppression by hairy vetch was 75 of that fallow soil (Teasdale and Mohler 2000), which is related to the light competition (Teasdale 1993) and the allelopathic compounds (Teasdale and Daughtry 1993; Hill *et al*. 2007). Biological N fixation (BNF) reduced the application of N fertilizers for subsequent crops (McVay *et al*. 1989; Clark *et al*. 1995; Kuo and Jellum 2002). The weed suppression and N supply by hairy vetch might contribute to better agronomic practices for sustainable agriculture and might minimize the production cost.

The objectives of this study were to observe biomass growth, N-fixing activity and nutrient accumulation of hairy vetch, as well as the influence of three different sowing times on hairy vetch growth in subtropical regions.

2.2.2. Materials and Methods

Experiment 1: Cultivation of hairy vetch

A pot experiment was conducted in subtropical Okinawa, Japan (26°N, 127°E), from November 2004 to June 2005. Hairy vetch (*Vicia villosa* Roth cv. Common; Yukijirushi Syubyo, Sapporo, Japan) was grown in 0.02 m^{-2} pots under outdoor conditions. Each pot was filled with 3.0 kg soil that was taken from the faculty farm in the University of the Ryukyus (Okinawa, Japan), as shown in Table 2.2.1. Each pot was fertilized to supply 0.5g P_2O_5 and 0.5g K_2O by using superphosphate and potassium sulfate, respectively.

Initially, five seeds of hairy vetch were sown on 15 November 2004. The plants were thinned to two plants per pot after 14 days of sowing (DAS). All measurements were performed with three replications. The dates of harvesting were 20 December 2004, 17 January, 7 February, 21 February, 7 March, 29 March, 18 April, 2 May, 23 May, and 27 June 2005. Growth performance of hairy vetch was shown in Picture 2.2.1.

Experiment 2: Cultivation of hairy vetch in different sowing dates

Hairy vetch (*Vicia villosa* Roth cv. Mametsuke, Yukijirushi Syubyo, Sapporo, Japan) seeds were sown on 17 October, November and December 2005 in a 0.02 m^2 pot filled with 2.2 kg of Akadama soil. Each pot was fertilized with 0.5g N (urea), 4.6g P_2O_5 (superphosphate) and 0.5g K_2O (potassium sulfate). The plants were grown in outdoor conditions. Initially, five seeds of hairy vetch were sown and inoculated with rhizobial inoculants by adding 12 g of soil that was taken from the faculty farm in the University of the Ryukyus and covered with vermiculite. The plants were thinned to two plants per pot at 14 DAS. The plants were harvested on 17 February (Picture 2.2.2), March (Picture 2.2.3) and April (Picture 2.2.4) in 2006. The measurements were performed with three replications in each harvesting date.

Plant and soil analysis

Immediately after sampling, N-fixing activity was measured by an acetylene reduction assay (ARA); thereby, the roots and nodules were put into plastic bags, 100 mL of acetylene and 900 mL of air were injected into the plastic bag, they were incubated for 1 h, and then 1 mL gas sample was injected into a gas chromatographer (GS-2; Sensortec, Tokyo, Japan) (Yoshida, 1982). The separate above-ground and underground parts of plants were dried at 70°C for 72 h and were weighed and powdered. Total carbon (C) and N concentrations were determined by a CHN Coder (JM10; G-Science Laboratory, Tokyo, Japan). The total P concentration was determined by calorimetric methods after drying ash at 450°C for 1h and dissolving it in 1:30 (v/v) diluted nitric acid (Hafner et al., 1993). The potassium (K), calcium (Ca) and magnesium (Mg) concentrations of all the samples were determined using an atomic absorption spectrophotometer (AAS) (Solaar 969; Japan Thermo Corporation, Tokyo, Japan) after Kjeldahl degradation.

The soils were air-dried and passed through a 2 mm sieve. The soil pH and electrical conductivity were measured in water, 1:2.5 and 1:5 (w/v), respectively. Total C and N concentrations were determined using a CHN Coder (JM10; G-Science Laboratory, Tokyo, Japan). The Inorganic N was determined by steam distillation after extracting with 2 mol L^{-1} KCl (Mulvaney 1996). The Ca-bound phosphate, as available phosphate, was determined by calorimetric methods after extraction with acetic acid. The phosphate absorption coefficient was determined by calorimetric methods after absorption with ammonium hydrogen phosphate. The exchangeable cation concentrations were determined by using an AAS after extraction with ammonium acetate. The chemical properties of the soil used in the above two experiments are shown in Table 2.2.1.

Statistical analysis

The data reported are mean values from three replicates with the standard error of the mean. The mean values of the biomass, N-fixing activity and nutrient uptake were analyzed by one-way analysis of variance with a completely randomize design. The comparison among the treatment means was evaluated using Fisher's Protected Least Significant Difference test, calculated at $P<0.05$. The statistical procedures were carried out with the software package, SPSS 14.0 for Windows (SPSS, Chicago, IL, USA).

2.2.3. Results

Experiment 1: Growth of hairy vetch in subtropical Okinawa

The biomass of the dry matter of the above-ground parts of hairy vetch increased up to 23 May 2005 and then decreased, whereas the underground (root and nodules) parts increased up to 2 May and thereafter remained constant (Fig. 2.2.1A).The ratio of the biomass of the above-ground part to the underground part (T/R ratio) of hairy vetch was 3.0.The ARA of hairy vetch showed a wide variation, from 10-30 μmol C_2H_4 h^{-1} pot^{-1}, during the growing period from January to May (Fig. 2.2.1B).

The fixed C and N uptake in both parts of hairy vetch had a similar pattern to that of the biomass growth (Fig. 2.2.2A, B). Furthermore, the fixed C peaked on 23 May, while the highest N uptake occurred on 18 April. The C/N ratio of the above-ground parts increased from 20 December 2004 to 27 June 2005, different from the underground parts, which increased from 20 December to 18 April and then stabilized at 15 (Fig. 2.2.2C).

The P and mineral uptake had a similar pattern to the biomass growth, except for the Mg uptake, which was higher in the underground parts than in the above-ground parts during the growing season from 18 April to 2 May (Fig. 2.2.3).The P uptake in the above-ground parts increased and achieved its maximum level on 29 March and then decreased. The underground parts achieved their maximum uptake level on 18 April. The K and Ca uptake in the above-ground parts peaked on 18 April, while the underground parts peaked on 2 May. The Mg uptake in the above-ground and underground parts peaked on 23 May and 18 April, respectively.

Experiment 2: Effect of three different sowing dates on the growth of hairy vetch

The biomass of the dry matter in the above-ground and underground parts of hairy vetch showed an increasing tendency with delayed harvest in March and April 2006 (Fig. 2.2.4), except when sown in October 2005 and harvested in April 2006 (Fig. 2.2.4A). Furthermore, the biomass in both parts increased significantly ($P < 0.05$) in March, except the underground parts of the plants sown in October. Moreover, the biomass of both parts decreased significantly in the April harvest when sown in October. In contrast, it showed a significant increase in the April harvest when sown in December. Flowering occurred in February for hairy vetch sown in October and November, while the plants sown in December was in its vegetative stage.

The N-fixing activity (ARA) of hairy vetch sown in December was significantly increased with delayed harvest in March (Fig. 2.2.4C). In contrast, the ARA decreased significantly in the April harvest when the plants were sown in October and November. The highest ARA resulted at 4 months after sowing (MAS), ranging from 22.1-28.1μmol C_2H_4 $h^{-1}pot^{-1}$, where no significant difference was found.

The fixed C and N uptake in the above-ground and underground parts of hairy vetch showed a similar pattern to that of the biomass growth at all sowing times and increased with delayed harvest in March (Fig. 2.2.5). However, when sown in October, the N uptake in the above-ground parts significantly decreased in April (Fig. 2.2.5B).The N uptake in both parts of the plants sown in December showed a significant increase in March. In addition, when sown in October, the N uptake in the above-ground parts of the plants was significantly higher in the February and March harvests than in those sown at other times. The C/N ratio of the plants tended to increase linearly with a delay in the March and April harvests, ranging from 8-17 at all sowing dates, except for the underground parts when sown in October and harvested in March. The C/N ratio of both parts of the plants in April showed significantly higher values in comparison with those plants that were harvested in February.

The P, Ca, and Mg uptake of both parts of hairy vetch at all sowing dates had a similar pattern of biomass yield for the plants that were harvested from February to April (Fig. 2.2.6). Moreover, the P and Mg uptake in both parts of the plants sown in October significantly decreased in April (Fig. 2.2.6A,D).The P uptake in both parts of the plants that were sown in December increased significantly with delayed harvest in March and April, while the Mg uptake in both parts of the plants sown in December increased significantly in March. However, the Ca uptake in the above-ground parts, when sown in October, did not decrease in April (Fig. 2.2.6C), in contrast with their biomass. The Ca uptake in the above-ground parts, when sown in November and December, showed a significant increase in March. Meanwhile, the Ca uptake of the underground parts of the plants sown in October and November showed a significantly higher uptake in March.

In addition, the K uptake in both parts of hairy vetch tended to decrease with a delay in harvest from February to April (Fig. 2.2.6B), except for the K uptake in the above-ground parts when sown in December and harvested in February. Moreover, when sown in October, the K uptake of the above-ground parts decreased significantly with delayed harvest in March and April, similar to that of the underground parts, which showed a significant decrease in the April harvest. When sown in November, the K uptake of the above-ground parts showed a significant decrease with delayed harvest in March and April and, when sown in December, there was a decrease at the April harvest. The P and mineral uptake of both parts of hairy vetch at 4 MAS showed a decreasing tendency with the sowing time from October to December, except that of the

Ca uptake when sown in October and harvested in February. The P, K, and Mg uptake in the above-ground parts was significantly higher when sown in October and harvested in February compared with the other treatments, while the Ca uptake was higher when sown in November and harvested in March. In addition, the K and Mg uptake in the underground parts was significantly higher when sown in October and harvested in February, while the Ca uptake was higher when sown in November and harvested in March.

2.2.4. Discussion

The biomass growth in the above-ground and underground parts of hairy vetch grown in subtropical Okinawa was higher in May by 1.3-fold and 2.9-fold, respectively, compared to hairy vetch grown in temperate regions, according to our previous study (Kitou *et al*. 2004). It also was found that hairy vetch in Okinawa grows more rapidly than that found in temperate regions. The higher and rapid biomass growth in subtropical Okinawa might have been related to the higher average temperature (less than 15°C), which is more favorable than that in temperate regions, where less than10°C from mid-December to mid-March is common (Japan Meteorological Agency 2007). Low temperature stress might directly affect the physiological processes, such as leaf area production, N fixation, and N distribution, in hairy vetch and similar results were found for white clover (Kessler *et al*. 1990; Castle *et al*. 2006; Brandsaeter *et al*. 2008). In addition, hairy vetch could emerge and establish well when sown in October, when the temperature ranged from 25-30°C, which was higher than the recommended temperature (Mosjidis and Zhang 1995). Therefore, the cultivation period of hairy vetch in subtropical regions is longer than that in temperate regions.

The biomass in both parts of hairy vetch tended to increase at 5 MAS at all sowing dates. The increase in biomass occurred in the vegetative and flowering stages before leaf senescence. In fact, hairy vetch sown at the early sowing date (in October) obtained the highest above-ground biomass when harvested in March. Moreover, when sown in October and harvested in April, the decrease in the biomass of both parts of hairy vetch at 6 MAS might be related to the late maturity stage. The flowering was not affected by the sowing date for hairy vetch sown in October and November when it was in the flowering stage in February. In contrast, delaying the sowing time in December might affect the seed emergence, N-fixing activity, and initial growth.

Furthermore, the high N uptake during the flowering stage of hairy vetch when sown in October and November might be related to the high N-fixing activity. The C/N ratio in both parts of hairy vetch was less than 20, similar to the findings that were reported from individual and mixed cultures (Kuo and Sainju 1998).

Despite no significant differences, the P and mineral uptake of hairy vetch tended to increase after the flowering stage, with the exception of the plants that were harvested in April and sown in October and November, where many leaves senesced to the ground.The higher Ca uptake in the above-ground parts when the plants were sown in October and harvested in April was related to the enhanced Ca concentration in the plants (data not shown). In contrast, the K uptake tended to decrease after the flowering stage, as reported previously (Caballero *et al*. 1996; Sainju *et al*. 1998).

In the present study, the above-ground biomass of hairy vetch under pot conditions, when converted to field conditions in subtropical Okinawa according to our previous study (Anugroho and Kitou 2008), obtained a positive correlation coefficient in the biomass (r^2 = 0.83) and potential weed suppression (r^2 = 0.82). Therefore, the biomass estimation of hairy vetch in the field ranged from 1755-4158 kg ha^{-1}, and resulted percentage inhibition of weeds in at least 56 of those fallow soils. Teasdale and Mohler (2000) revealed that the percentage inhibition of weed emergence more than 75 is consistently achieved only when the mulch biomass is more than 8000 kg ha^{-1} and the mulch thickness is more than 10 cm. Most cover crops, such as rye and hairy vetch, produce a natural level of biomass of 3000–5000 kg ha^{-1}.The weed suppression in untilled cropping systems provided by a cover crop can be varied according to the cover crop species, residue biomass, and weed species (Teasdale 1998; Liebman and Mohler 2001).The residue must be present in very high amounts to provide a high level of physical suppression of annual weeds in subtropical regions; for example, *Bidens pilosa*, a dominant weed that contains phytotoxins (Deba *et al.* 2007). In addition, the release of phytotoxicity from methanol and ethyl acetate in hairy vetch residue, which contains allelopathic compounds, might inhibit weed roots and hypocotyl growth (Hill *et al.* 2007).

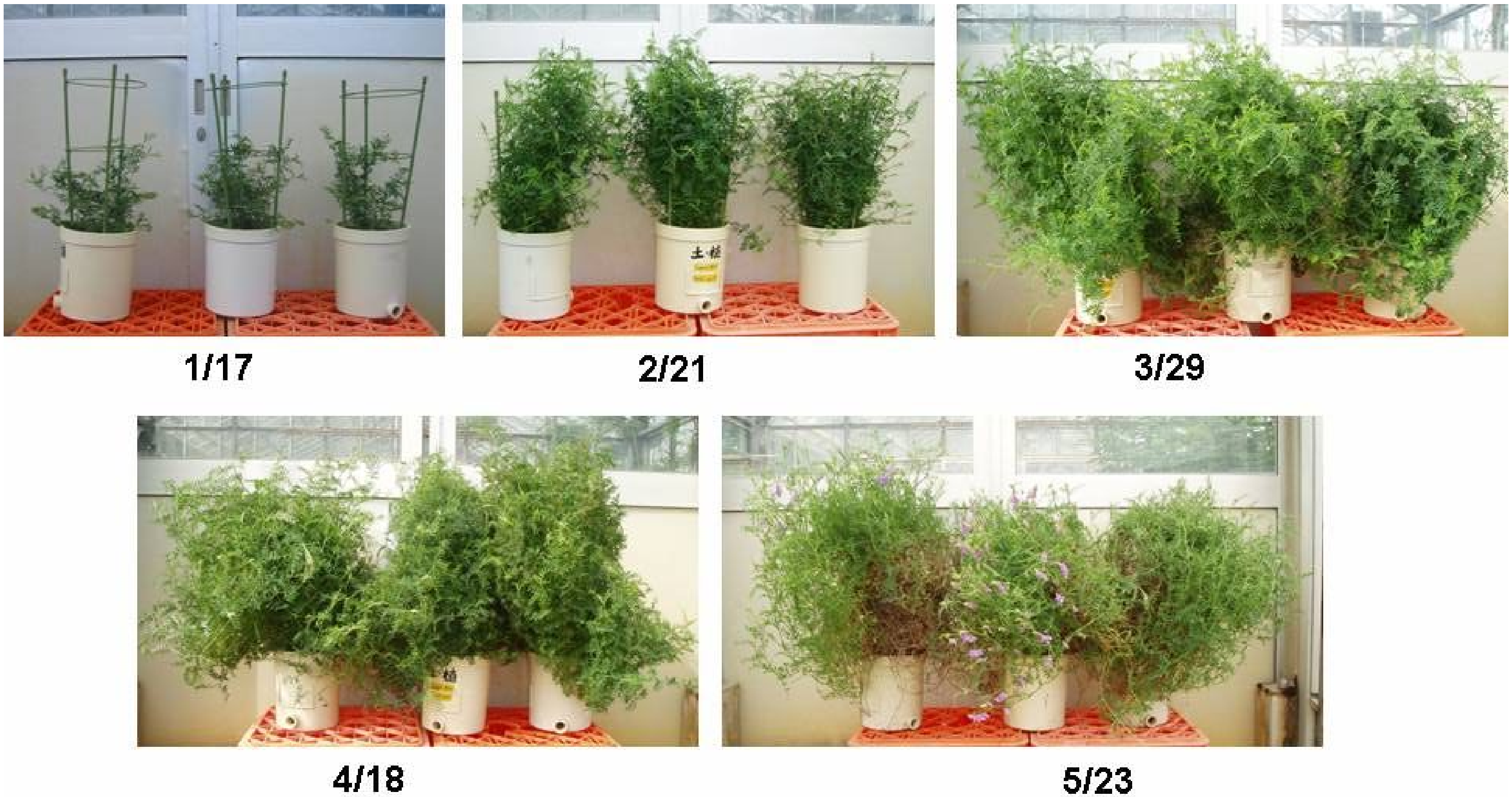
Experimental results showed that, in subtropical Okinawa, hairy vetch as a green manure can contribute higher N; similar results were found in a corn cropping system conducted in a temperate region (Kuo and Jellum 2002). The N content of hairy vetch substantially reduced the N fertilizer requirement for high N-requiring crops, such as corn. The biomass production of hairy vetch in subtropical Okinawa might substitute the N fertilizer requirement by 68–138 kg N ha^{-1}, according to our previous study (Anugroho and Kitou 2008), with a high correlation coefficient (r^2 = 0.83). Teasdale and Mohler (2000) reported that the biomass production of hairy vetch in temperate regions varied in the range of 3000–5000 kg ha^{-1}, which might substitute the N fertilizer requirement by 100-150 kg N ha^{-1}, mainly from BNF. Meanwhile, the recommended dose of N fertilizer in subtropical regions for a corn cropping system was 100 kg N ha^{-1} (Zougmore *et al.* 2006).A low C/N ratio of 10–14 could increase the mineralized N (Kuo and Jellum 2002). A low C/N ratio also improved soil moisture infiltration and conservation (McVay *et al.* 1989; Clark *et al.* 1995) and controlled pests and diseases (Rothrock *et al.* 1995).

The biomass growth and nutrient uptake of hairy vetch cultivated in subtropical Okinawa gave similar results compared with that cultivated in temperate regions. The high biomass production ability of hairy vetch can increase the organic matter and N content in the soil (Seo *et al*. 2006). The organic matter in subtropical region soils decomposed five times faster than that in temperate regions (Sanchez and Logan 1992).The decomposition of organic matter derived from legume plants could be stimulated by tillage operations (Thonnissen *et al*. 2000).Therefore, low levels of organic residues were insufficient to inhibit weed emergence. However, hairy vetch residue left on the soil surface in untilled systems can suppress weed emergence (Teasdale and Mohler 2000). In our present study, the wide range of cultivation periods of hairy vetch in subtropical regions can contribute to various cropping systems. Hairy vetch can be sown from October to December to cover the fields and suppress weeds effectively in the winter and the spring seasons. The nutrient supplement from hairy vetch as a green manure should be met with the nutrient requirement of the subsequent crop, particularly N. Some researchers reported that, in corn cropping systems, the high biomass of hairy vetch can cover the field until March or April and can input high N levels into soils (Seo *et al*. 2006; Zougmore *et al*. 2006). Furthermore, future work should be carried out to study the growth of hairy vetch cultivated in subtropical fields in order to investigate its effect on weed biomass and the physical, chemical, and biological properties of soils in subtropical regions.

Table 2.2.1. Soil chemical properties used in two separate experiments

		Experiment 1	Experiment 2
pH		6.9	5.8
EC (10^{-3}S m^{-1})		14.7	12.3
Total Carbon (×10^{-3} kg kg^{-1})		11.6	13.6
Total Nitrogen (×10^{-3} kg kg^{-1})		1.3	1.2
C/N		8.8	11.9
Inorganic N (×10^{-6} kg kg^{-1})		18.3	15.5
Available P_2O_5[a)] (×10^{-6} kg kg^{-1})		nd[b]	nd
P_2O_5 Absorption Coefficient (×10^{-3} kg kg^{-1})		8.6	21.1
Exchangeable Cation (×10^{-3} mol_c kg^{-1})	K	6.7	9.3
	Ca	166.0	48.4
	Mg	14.2	7.0
	Na	5.4	0.5

[a)] 0.025 LL^{-1} acetic acid extractable P_2O_5
[b)] nd: no detection



Picture 2.2.1. Growth performance of hairy vetch cv. Common from January to May 2005.

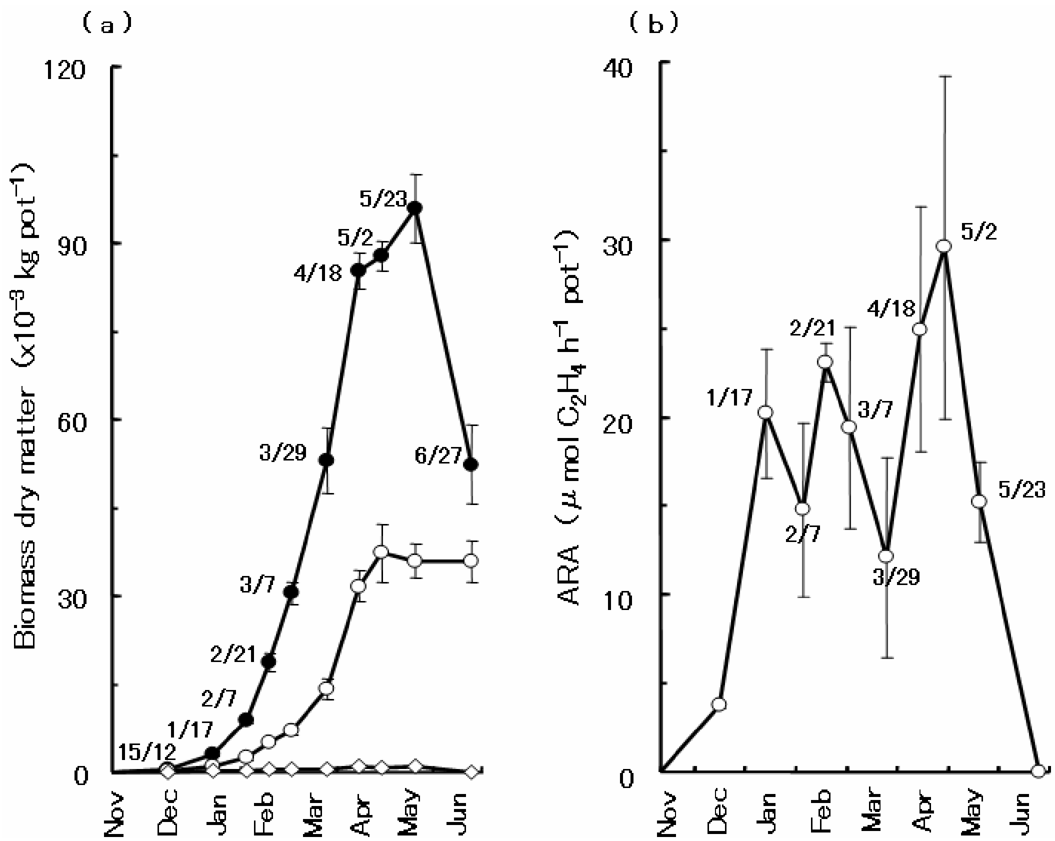


Fig. 2.2.1. Biomass of the dry matter (a) in the top (●), root (○) and nodule (◇) parts and N-fixing activity (b) of hairy vetch from November 2004 to June 2005. The vertical bars represent standard deviations (n = 3). ARA, acetylene reduction assay.

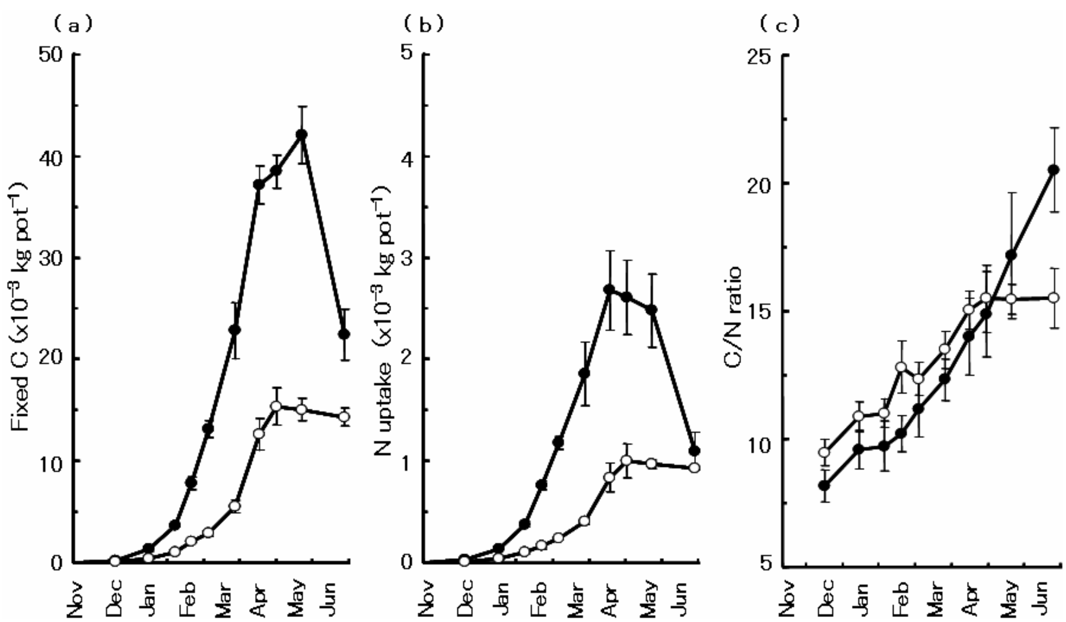


Fig. 2.2.2. (a) Fixed C, (b) N uptake and (c) C/N ratio in above-ground (●) and underground (○) parts of hairy vetch from November 2004 to June 2005. The vertical bars represent standard deviations (n = 3).

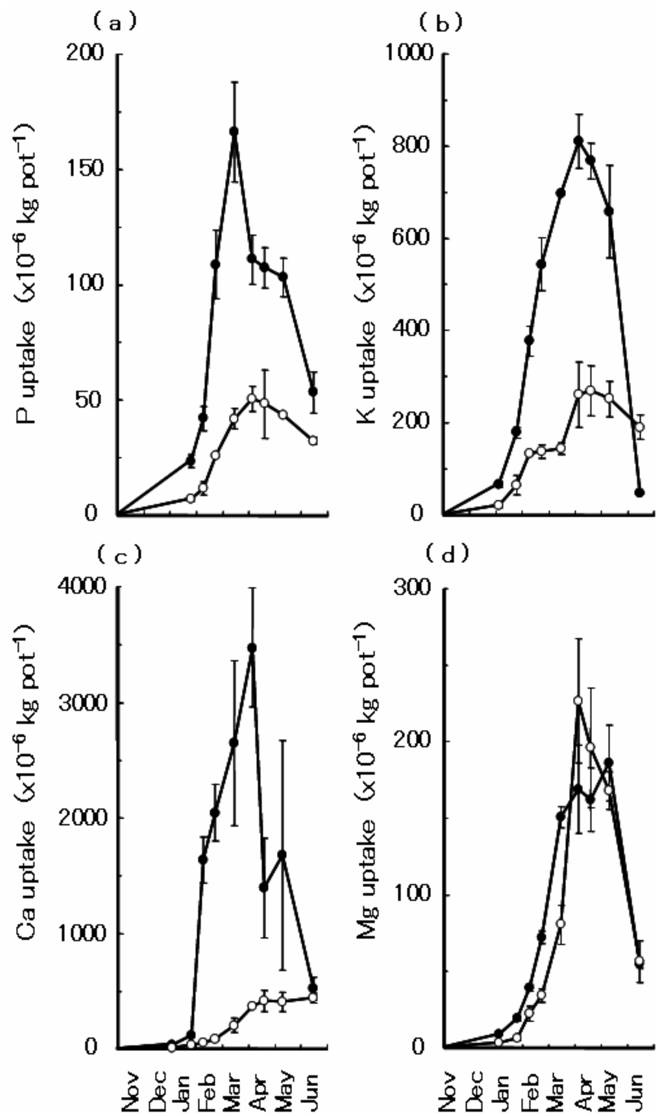
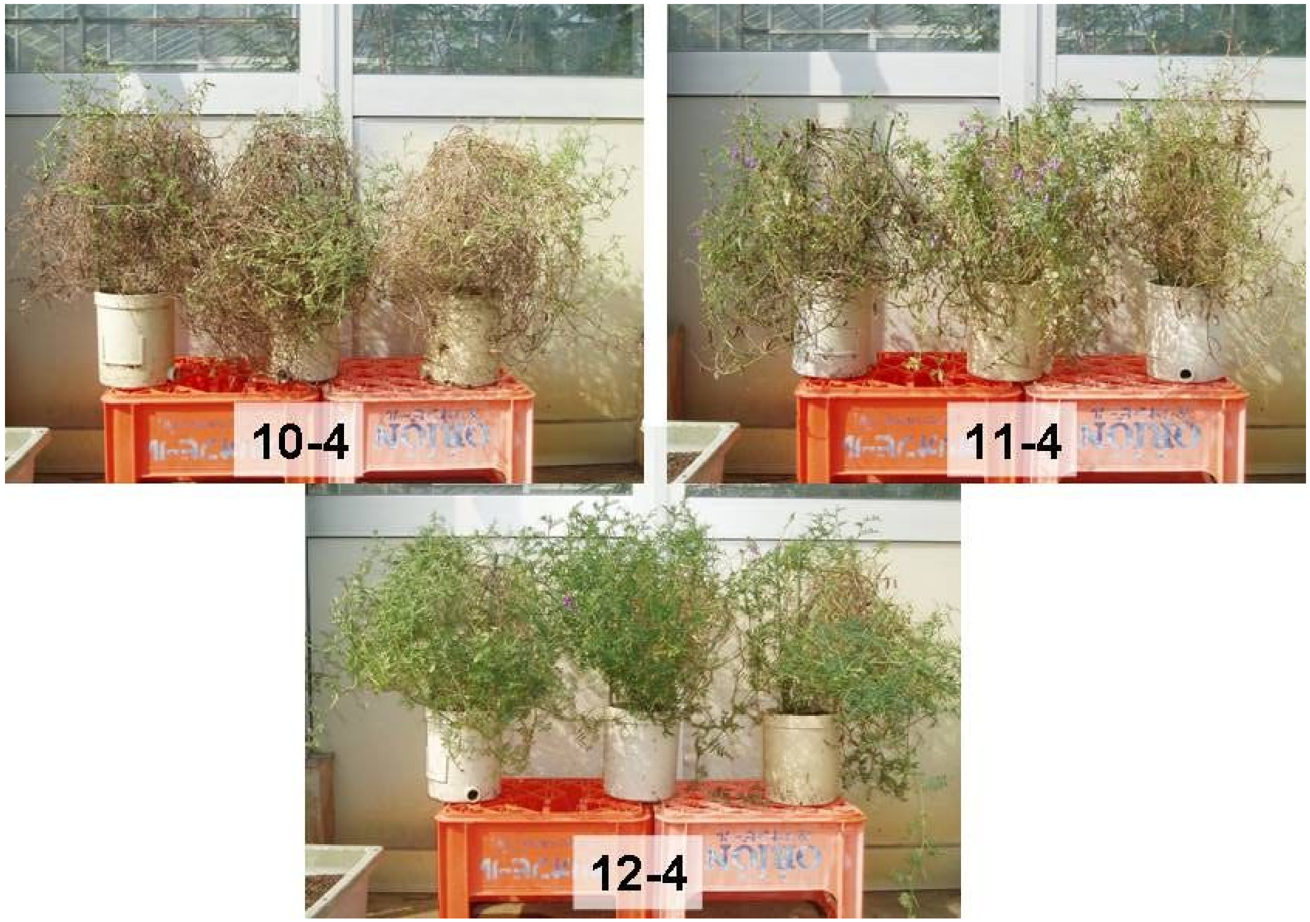


Fig. 2.2.3. Uptake of (a) P, (b) K, (c) Ca and (d) Mg in the above-ground (●) and underground (○) parts of hairy vetch from November 2004 to June 2005. The vertical bars represent standard deviations (n = 3).

Picture 2.2.2. Growth performance of hairy vetch cv. Mametsuke in three different sowing dates harvested in February 2006.

Picture 2.2.3. Growth performance of hairy vetch cv. Mametsuke in three different sowing dates harvested in March 2006.



Picture 2.2.4. Growth performance of hairy vetch cv. Mametsuke in three different sowing dates harvested in April 2006.

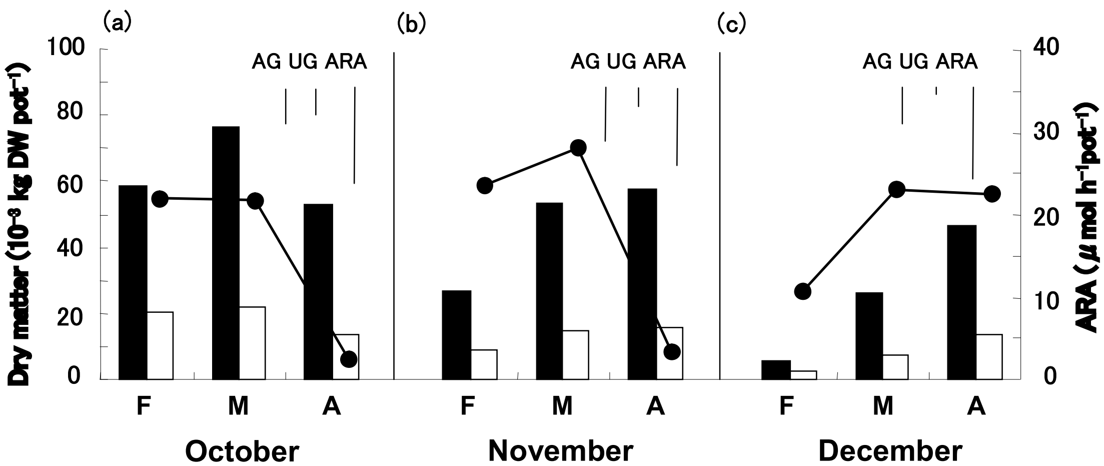


Fig. 2.2.4. Biomass of the dry matter in the above-ground (AG and ■) and underground (UG and □) parts and N-fixing activity (acetylene reduction activity [ARA] and ●) of hairy vetch with the different sowing dates (a) October; (b) November, and (c) December and the harvest dates February (F), March (M), and April (A). The vertical bars represent the Least Significant Difference ($P < 0.05$).

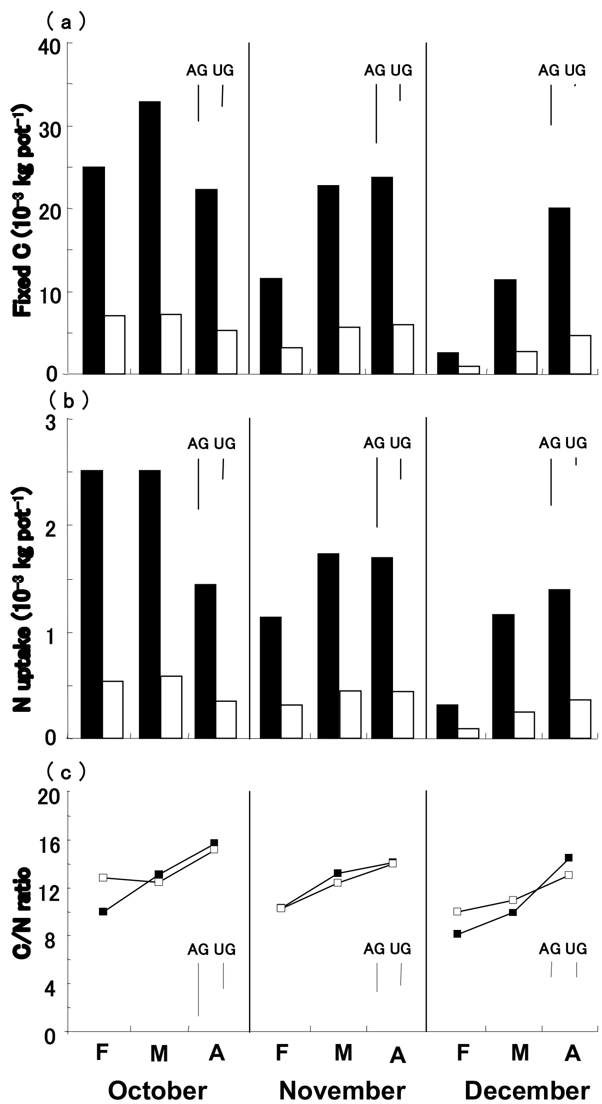


Fig. 2.2.5. (a) Fixed C, (b) N uptake, and (c) C/N ratio in the above-ground (AG and ■) and underground (UG and □) parts of hairy vetch with the different sowing dates (a) October; (b) November, and (c) December and the harvest dates February (F), March (M), and April (A). The vertical bars represent the Least Significant Difference ($P < 0.05$).

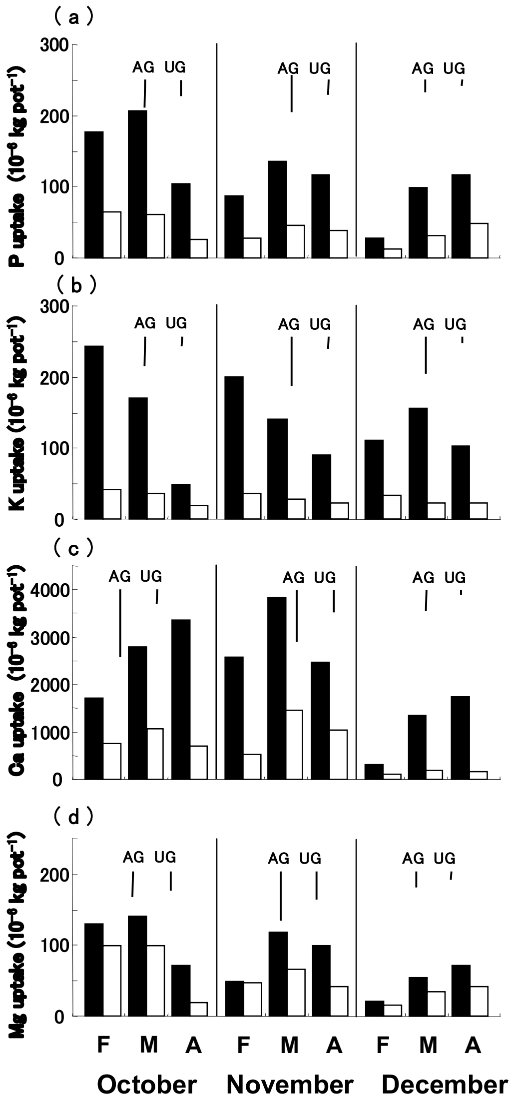


Fig. 2.2.6. Uptake of (a) P, (b) K, (c) Ca and (d) Mg in the above-ground (AG and ■) and underground (UG and □) parts of hairy vetch with the different sowing dates (a) October; (b) November, and (c) December and the harvest dates February (F), March (M), and April (A). The vertical bars represent the Least Significant Difference ($P < 0.05$).

2.3. Growth and Nutrient Uptake of Field-grown Hairy Vetch, Weed Suppression and Soil Chemical Properties as Influenced by Sowing and Harvesting Date

2.3.1. Introduction

Integrated weed management by using cover crops has reduced the emergence and competition of weeds through living mulch (McLenaghen et al 1996; Liebman and Davis 2000; Zoschke and Quadranti 2002). In addition, weed growth can be inhibited by the cyanamide, methanol and ethyl acetate contained in hairy vetch (*Vicia villosa* Roth) residue, together with competition for light, nutrients and water (Kamo et al. 2003; Hill et al. 2007).

The use of hairy vetch as a cover crop in temperate regions has reduced the percentages of the weed density and weed biomass by 70-78 and 52-70, respectively, compared with fallow treatments (Teasdale and Daughtry 1993). In addition, the inhibition percentage of weed emergence more than 75 is consistently achieved only when the mulch biomass and mulch thickness of hairy vetch exceeds 8000 kg ha^{-1} and 0.1 m, respectively (Teasdale and Mohler 2000). Hairy vetch produces a natural level of biomass of 3000-5000 kg ha^{-1}, with the associated N content ranging from 100 to 150 kg ha^{-1}.

In our previous pot study, hairy vetch that was sown from October to December in subtropical climate conditions showed a higher biomass and nutrient uptake from February to April (Anugroho et al. 2006). This evidence might indicate that hairy vetch can be grown in the agricultural fields in subtropical regions to suppress weeds and to improve soil fertility, which also is practised in temperate regions. However, hairy vetch grown in pot might not explain its actual effect on weed suppression and the improvement of soil fertility under field conditions. Thus, information is needed on the effect of hairy vetch in the field in subtropical regions, and its influence on soil chemical properties.

The present field study conducted to investigate the influence of sowing date and harvesting date on the biomass growth and nutrient uptake of hairy vetch, its weed suppression and the soil chemical properties in subtropical regions. This information will aid to determining the available N in order to estimate the N requirement of the subsequent crop in subtropical regions.

2.3.2. Materials and Methods

Plant growing

The study was conducted at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. The soil type utilized in the experiment was dark red soil ("Shimajiri Mahji"). The chemical properties of the soil such as pH, EC, total C, total N, total P_2O_5 and P_2O_5 absorption coefficient were 6.0, 7.95 x10^{-3}S m^{-1}, 15.3 x10^{-3}kg kg^{-1}, 1.8 x10^{-3}kg kg^{-1}, 28.5 x10^{-3}kg kg^{-1}, 9.6 x10^{-3}kg kg^{-1}, respectively. The exchangeable cation of K, Ca, and Mg were 25.8, 73.5 and 10.9 x$10^{-3}$$mol_c$ kg^{-1}, respectively.

Hairy vetch (*Vicia villosa* Roth cv. Mameko, Kaneko Syubyo) was sown on 20 October (HV+10) and December (HV+12) in 2006. The fallow condition was without the sowing of hairy vetch (HV-F). All the treatments were conducted in plots of 3 m x 7 m with three replications and a completely randomized block design. All the plots were plowed by rotary tiller. Hairy vetch was sown manually, with 0.25 m interrow and intrarow spacing and five plants per hill, giving a plant density of 80 plants m^{-2}. The weeds were allowed to grow naturally in all the plots during the experiment. The plants and soils were sampled in 0.5 m x 0.5 m quadrats with a 0.10 m soil depth. Hairy vetch was harvested on 20 February, 20 March, 20 April and 20 May 2006 (Picture 2.3.1). The weeds were sampled on 30 May 2007. The soil in each plot was sampled seven times during the period from 20 February to 20 August 2007.

Plant and soil analysis

The separated top and root parts of the plants were dried at 70°C for 72 h, weighed, and then powdered. The total C and N concentrations were determined by a CHN coder (JM10; G-Science Laboratory Company, Tokyo, Japan). The total P concentration was determined by calorimetric methods after drying the ash at 450°C for 1h and dissolving it in 1:30 (v/v) diluted nitric acid (Hafner et al., 1993). The concentration of K, Ca and Mg was determined using an atomic absorption spectrophotometer (AAS) (Solaar 969; Japan Thermo Corporation, Tokyo, Japan) after Kjeldahl degradation.

The soils were air-dried and passed through a 2 mm sieve. The soil pH and EC were measured in water at 1:2.5 and 1:5 (w/v), respectively. The total C and N concentrations were determined using a CHN coder (JM10; G-Science Laboratory Company, Tokyo, Japan). The inorganic and available N were determined by steam distillation after extraction with 2 mol L^{-1} KCl (Mulvaney 1996). A 20g soil sample was incubated at 0.6 of the maximum water holding capacity and 30°C for 28 days prior to the determination of the available N (Matsumoto et al. 2000). Ca-bound P_2O_5, as the available phosphate, was determined by calorimetric methods after extraction with 0.025 L L^{-1} of acetic acid. The P_2O_5 absorption coefficient was determined by calorimetric methods after absorption with ammonium hydrogen phosphate. The exchangeable cations were determined by AAS (Solaar 969; Japan Thermo Corporation, Tokyo, Japan) after extracting with ammonium acetate.

Statistical analysis

The data are the mean values of three replicates with the standard error of the mean. The mean values of the biomass and nutrient uptake were compared by one-way analysis of variance (ANOVA) with a completely randomized block design. The comparison of the influence of two different sowing dates on the biomass and nutrient means were evaluated using Dunnett's *t*-test, at $P < 0.05$. The soil samples in the plots treated with hairy vetch were compared with the fallow plots using the Least Significant Difference (LSD) test, at $P <0.05$. The statistical procedures were carried out with the software package SPSS 14.0 for Windows (SPSS, Chicago, IL, USA).

2.3.3. Results

Effect of the sowing date on the biomass of hairy vetch

On both sowing dates, the biomass of the top parts (BOT) of hairy vetch increased from February to April and then decreased in May (Fig. 2.3.1A). Moreover, the BOT of hairy vetch sown in October and harvested in February, March and April was significantly higher than in May. Furthermore, the BOT of the plant sown in October and December and harvested in February, March and April showed a significant difference in comparison with those harvested in May. The 2 month delay in the sowing date led to BOT losses of 222 $x10^{-3}$kg m^{-2} in February, 129 $x10^{-3}$kg m^{-2} in March, 124 $x10^{-3}$kg m^{-2} in April, and 84 $x10^{-3}$kg m^{-2} in May. Meanwhile, the biomass of the root parts (BOR) of hairy vetch sown in October decreased from February to April, in contrast with the BOR of the plant sown in December (Fig. 2.3.1B). However, the BOR of the plant sown in October and December and harvested in February showed a significant difference in comparison with those harvested in March and April. The BOT and BOR of the plants grown during the experimental period varied from 140 to 400 $x10^{-3}$kg m^{-2} and 5 to 20 $x10^{-3}$kg m^{-2}, respectively.

Effect of mulching on weed biomass

Several weeds were found in the field during the spring season in subtropical Okinawa, with their order from dominant to rare: *Bidens pilosa*, *Amaranthus spinosus*, *Amaranthus viridis*, *Sonchus oleraceus*, *Sonchus asper*, *Chenopodium album*, *Cyperus rotundus*, *Digitaria ciliaris*, *Digitaria timorensis*, *Oxalis corymbosa*, *Rottboellia exaltata* and *Solanum nigrum*. The weed biomass of HV+10 and HV+12 was significantly lower ($P<0.05$, LSD) than that of HV+F (Fig. 2.3.2). The weed biomass percentages of the HV+10 and HV+12 plots were 37.2 and 64.8, respectively compared with the HV-F plots, with 100 of weed biomass.

Nutrient uptake of hairy vetch

The fixed C of the top parts of hairy vetch sown in October and December showed a similar pattern to that of the biomass (Fig. 2.3.3A). The fixed C of the plants sown in October and December reached approximately 175 $x10^{-3}$ kg m^{-2} and 120 $x10^{-3}$kg m^{-2}, respectively. In addition, the N uptake of the top parts of hairy vetch was a relative constant in the March harvest when sown in October, but it increased when sown in December. Then, it decreased in the April and May harvests when hairy vetch was sown in October and December (Fig. 2.3.3B). The N uptake of the plants sown in October and December was significantly lower with a delay in the May harvest than compared with a delay in the March and April harvests. The N uptake of the plants sown in October and December varied in the range of 5-14 $x10^{-3}$kg m^{-2} and 3-10 $x10^{-3}$kg m^{-2}, respectively. The C/N ratios of hairy vetch sown in October increased from February to May, when sown late in December, they increased from March to May; thus, their ratios ranged from 8.8 to 19.4 (Fig. 2.3.3C).

Generally, the P and cation uptake in the top parts of hairy vetch sown in October showed a decrease with a delay in harvesting from February to May, except for the Ca uptake (Fig. 2.3.4). Meanwhile, for hairy vetch sown in December, they increased in

March, and then decreased in April and May (Fig. 2.3.4A, B, D), except for the Ca uptake, which increased from February to April (Fig. 2.3.4C). Moreover, the P, K and Mg uptake in February, and the Ca uptake in both February and March by the top parts of hairy vetch were significantly higher for the October planting than for the December planting. The P, K, Ca, and Mg uptake of hairy vetch sown in October and December varied from 240 to 1251 $x10^{-6}kg\ m^{-2}$, 1464 to 9452 $x10^{-6}kg\ m^{-2}$, 803 to 2761 $x10^{-6}kg\ m^{-2}$ and 222 to 895 $x10^{-6}kg\ m^{-2}$, respectively.

Effect of mulching on the soil chemical properties

The soil inorganic N is mostly in the form of ammonium (NH_4^+-N) and nitrate (NO_3^--N). The soil inorganic N content for HV+10 and HV+12 was calculated by the sum of the NH_4^+-N and NO_3^--N contents (Fig. 2.3.5A,B). It was revealed that the soil inorganic N content for HV+10 and HV+12 was generally higher than that for HV-F from March to June. The NH_4^+-N content for HV-F decreased from February to June, while for HV+10, it was increased from February to March, but decreased in April and May. It then increased in June, decreased in July, and increased again in August. For HV+12, the NH_4-N content increased from February to March, but only slightly increased from April to June. It then decreased in July and increased again in August (Fig. 2.3.5A). Meanwhile, the NO_3^--N content in HV+10 and HV+12 increased from February to May, in contrast with that of HV-F (Fig. 2.3.5B). Then, the NO_3^--N content in HV+10 decreased from May to July and became constant from July to August. However, for HV+12, the NO_3^--N content decreased from May to June and became constant from June to August. Moreover, the NO_3^--N content for HV+10 sampled in April and May was significantly higher than that for HV-F.

The available N in the soils with and without hairy vetch is shown in Fig. 2.3.5C. The available N was higher for HV+10 and HV+12 from February to July than that for HV-F, except for HV+12 in March and June. Moreover, the available N of HV+10 in February, March and April was significantly higher than that for HV-F.

The available P_2O_5 and exchangeable cations in the soils with and without hairy vetch mulch are shown in Fig. 2.3.6. The available P_2O_5 that was sampled in June was significantly higher for HV+12 than for HV+10 and HV-F (Fig. 2.3.6A). Similarly, the available P_2O_5 that was sampled in August was significantly higher for HV+10 and HV+12 than for HV-F. The exchangeable K^+, Ca^{2+} and Mg^{2+} were not significantly affected by hairy vetch mulching.

2.3.4. Discussion

In this study, the BOT of hairy vetch reached approximately 4000 kg ha^{-1} when sown in October and harvested from February to April in subtropical regions. The BOT was reduced by more than 1000 kg ha^{-1} when sown in December. The relatively low biomass of hairy vetch sown in December and harvested in February (vegetative stage) in subtropical Okinawa may be related to the low temperature at the initial growth stage. A favorable condition for plant growth in subtropical Okinawa prevails in October, when the initial temperature of 25°C slowly decreases to less than 20°C in December. Thus, the early growth of hairy vetch sown in December was inhibited by the temperature decreasing to 15°C in late January (Japan Meteorological Agency, 2007).

The 2 month delay of the sowing date in subtropical Okinawa reduced the biomass percentages of hairy vetch by 60 and 30 of that harvested at its vegetative (February) and flowering (March and April) stages, respectively. The biomass reduction, related to the delay in sowing date in subtropical Okinawa, was higher than previously reported in temperate regions by Teasdale et al. (2004). The biomass reduction percentages were found to be approximately 42 when harvested in the vegetative stage and 20 when harvested in the flowering stage. The delay sowing date by 2 months in subtropical

Okinawa can reduce weed suppression percentage by 30 at the desiccated stage, when the weeds were dominated by *Bidens pilosa*. In temperate regions, Teasdale and Doughtry (1993) reported that a living mulch of hairy vetch more effectively suppressed weeds than at its desiccated stage. In addition, the release of phytotoxic compounds from hairy vetch during the vegetative stage might be one of the potential inhibitors that suppressed the weeds (Kamo et al. 2003; Hill et al. 2007).

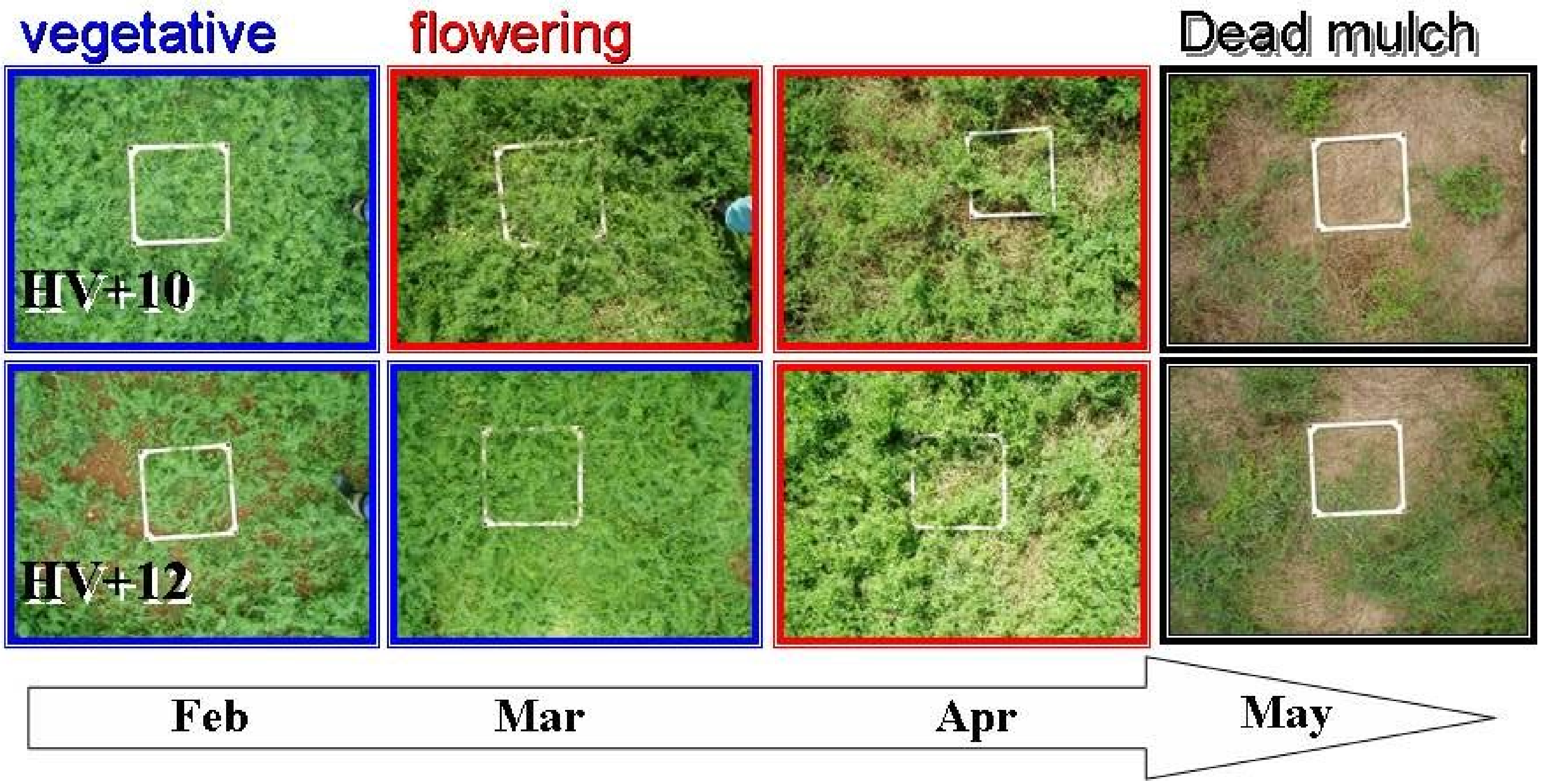
The N production level of the top parts of hairy vetch grown in subtropical Okinawa reached approximately 140 kg ha^{-1}. It was similar to the data reported previously by Teasdale et al. (2004) in temperate regions. This level of N production is sufficient to meet most of the N requirements of tomato and corn (Abdul-Baki and Teasdale 1993; Zougmore et al. 2006). The N uptake of hairy vetch in subtropical Okinawa was affected by the sowing date, where a high N uptake was found when the plants were sown in October and harvested in February and March. This might have been related to the late vegetative and early flowering stages of hairy vetch. In both stages, high N concentration levels of hairy vetch were observed. However, the favorable temperature and soil moisture content for hairy vetch sown in late summer or early fall might affect its seed germination and establishment. This result was similar to previous findings by Teasdale et al. (2004). In subtropical Okinawa, C/N ratio of hairy vetch at the early, mid and late harvest was less than 20. Several researchers have observed that C/N ratio of hairy vetch was less than 25, which is a limitation that determines N mineralization or immobilization from the plant residue (Wagger 1989; Vigil and Kissel 1991). Similarly, the high P and mineral uptake of hairy vetch, when harvested in February, might be related to the high uptake of P and minerals in the late vegetative and early flowering stages.

Previously, this field had been used for cash crop production such as turmeric and sweet potatoes during the summer season, without the addition of green manure. The production of turmeric needs a long cultivation period, from May to February. Meanwhile, sweet potatoes are cultivated during the summer season and the field was left bare from winter season to spring season. Generally, weeds occupied the field before the sowing of subsequent crops. During the continuous crop production or bare conditions, the soil was gradually degraded and had a low organic residue with higher weed infestation and low soil moisture. It needed highly expensive chemical fertilizer before the planting of a cash crop. In recent years, the use of chemical fertilizer has been reduced to prevent groundwater pollution and to enhance sustainable agriculture. Hairy vetch, with early and late sowing dates, improved the field soil conditions in June, 1 month after the plants died. It was clear that the pH and exchangeable Ca and Mg in the soils increased when hairy vetch sown in October, but they decreased when hairy vetch was sown in December. The inorganic N increased in the soils with a living mulch of hairy vetch, in contrast to the EC and exchangeable K. The depletion of soil elements might gradually recover through the incorporation of a high biomass of hairy vetch into the soils.

The organic N derived from hairy vetch residue can be mineralized into NH_4^+-N and NO_3^--N forms. The NO_3^--N content in the soils did not decrease, even though hairy vetch absorbed much of its inorganic N, mostly in the form of NO_3^--N. This indicated that the hairy vetch residue had been mineralized and increased the level of NO_3^--N in the soils. The increase of NO_3^--N in the soils might be related to the high N fixation of hairy vetch (Mueller and Thorup-Kristensen 2001; Anugroho et al. 2006; Choi and Daimon 2008). Furthermore, the early sowing date of hairy vetch with a delayed harvesting date can increase the level of NO_3^--N in soils.

The growth of hairy vetch in subtropical Okinawa was affected by the sowing date. Hairy vetch should be sown in the period from mid-October to early November because of the favorable conditions for its initial growth prevails when temperature ranges from

20-25°C. Thereby, it produces a high BOT and suppresses the weeds physically in the winter and the spring seasons. In addition, N accumulation in the top parts of hairy vetch can supply higher inorganic N and available N into the soils through the mineralization process. However, the soils left bare after incorporation of hairy vetch can stimulate weed emergence and establishment because of high N mineralization and availability. Further studies should be conducted to evaluate the effects of living mulch and the incorporation of hairy vetch on the growth and yield of a subsequent crop in subtropical regions.



Picture 2.3.1. Growth performance of field-grown hairy vetch cv. Mameko in two different sowing dates harvested from February to May 2007.

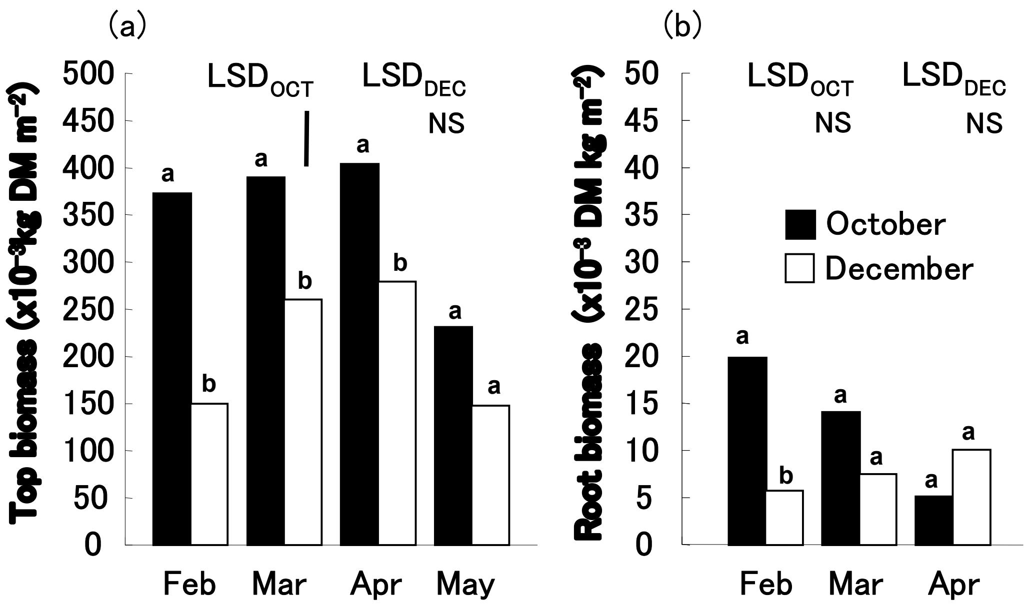


Fig. 2.3.1. Biomass of the top and root parts of hairy vetch sown in October and December. The different letters represent a significant difference for each harvesting date, according to the *t*-test ($P<0.05$). The vertical bar shows a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test ($P < 0.05$). DM, dry matter; NS: no significant difference.

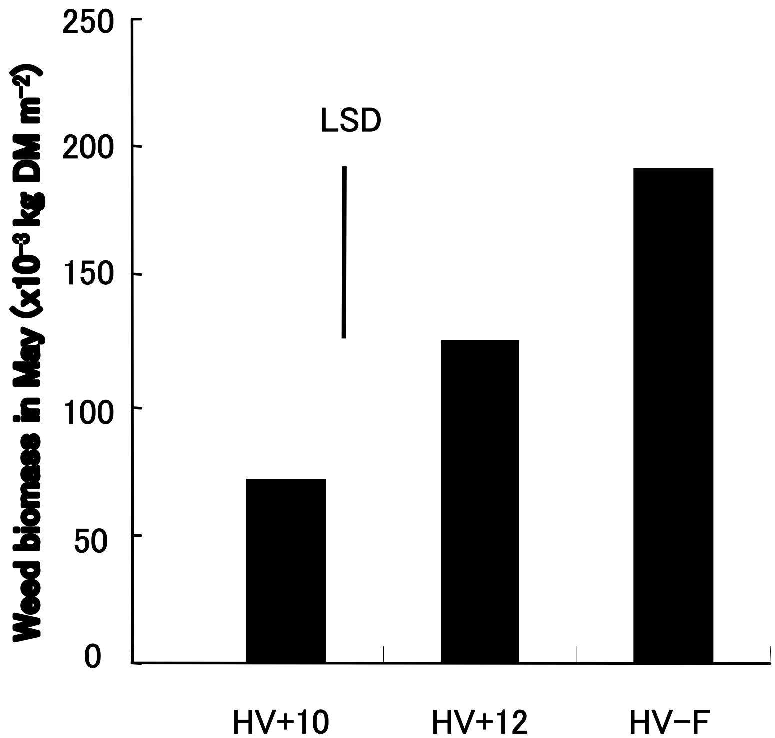


Fig. 2.3.2. Weed biomass in May with hairy veth sown in October (HV+10) and in December (HV+12), and without hairy vetch (HV-F). The vertical bar shows a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test ($P < 0.05$). DM, dry matter.

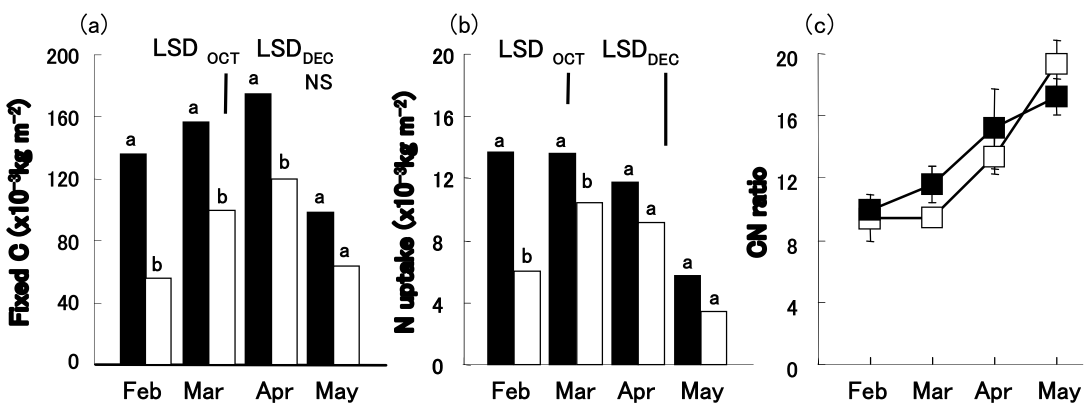


Fig. 2.3.3. Fixed-C, N uptake and CN ratio in the top parts of hairy vetch sown in October (■) and December (□). The different letters represent a significant difference for each harvesting date, according to the *t*-test ($P<0.05$). The vertical bars show a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test ($P < 0.05$). NS: no significant difference.

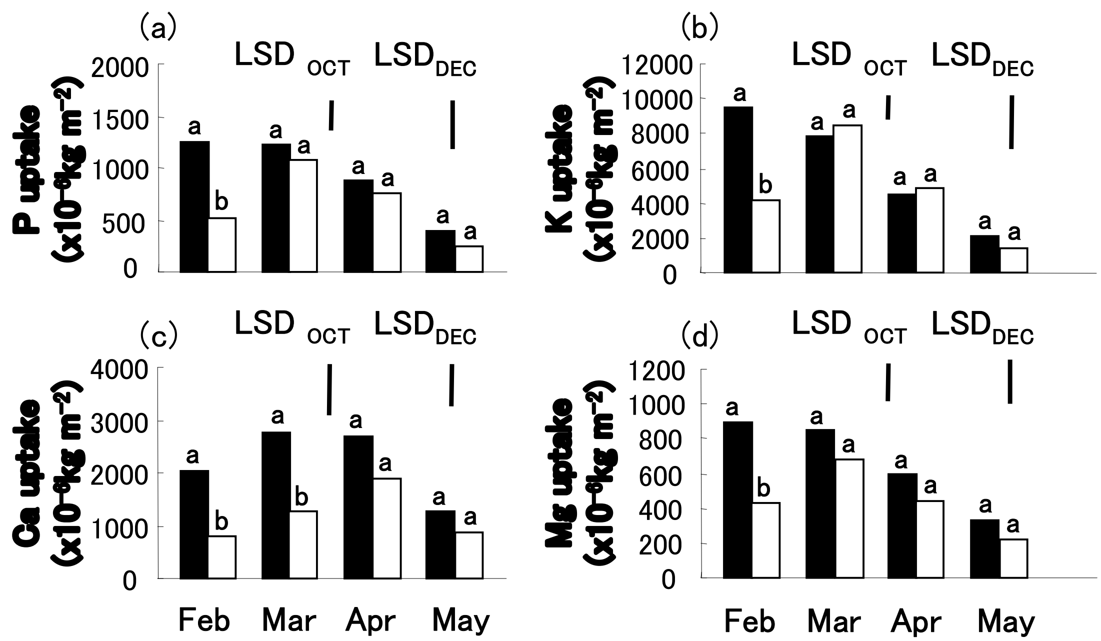


Fig. 2.3.4. Uptake of P, K, Ca and Mg in the top parts of hairy vetch sown October (■) and December (□). The different letters represent a significant difference for each harvesting date, according to the *t*-test (P<0.05). The vertical bars show a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test (P < 0.05). NS: no significant difference.

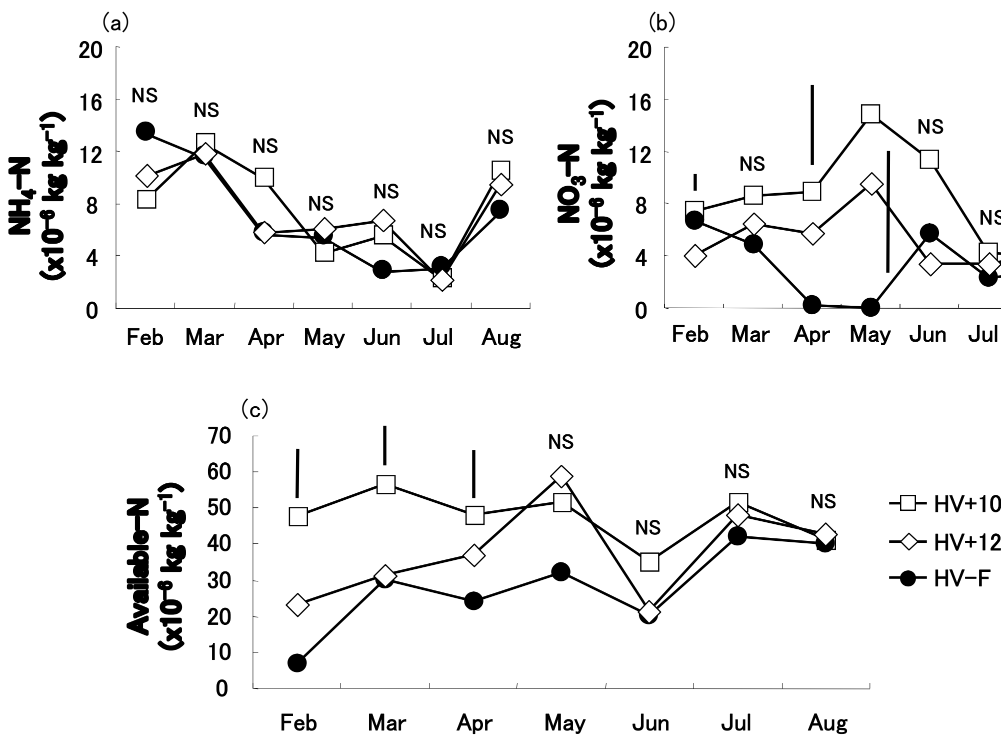


Fig. 2.3.5. Ammonium (NH_4-N), nitrate (NO_3-N) and available N in the soil with hairy vetch sown in October (HV+10), in December (HV+12), and without hairy vetch (HV-F). The vertical bars show a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test ($P < 0.05$). NS: no significant difference.

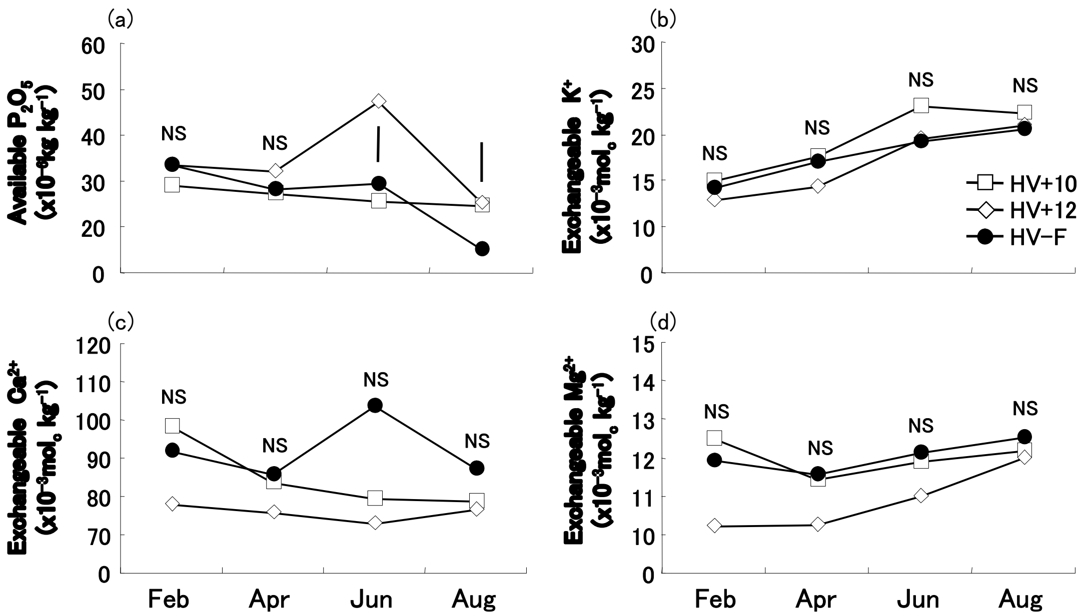


Fig. 2.3.6. Available phosphate (P_2O_5) and exchangeable cations of K, Ca and Mg in the soil with hairy vetch sown in October (HV+10), in December (HV+12), and without hairy vetch (HV-F). The vertical bars show a significant difference among the harvesting time, according to the Least Significant Difference (LSD) test ($P < 0.05$). NS: no significant difference.

2.4. Effect of Cover Cropping and Subsequent Incorporation of Field-grown Hairy Vetch on Weed Suppression and Soil Inorganic N

2.4.1. Introduction

The inclusion of a fallow period in subtropical cropping systems results in development of a weed population and nutrient losses from the soil profile by leaching (Zougmore et al. 2006). Weeds that develop in the fallow field can harbor insects, disease, and nematode pests that are harmful to a subsequent crop (Derr 2004). On the Nutrients may be lost from agricultural fields by runoff, leaching, and volatilization, particularly during the rainy season.

Weed suppression and raising organic-N content are two of the many benefits of including legume cover crops in a fallow cropping system. Utilization of hairy vetch (*Vicia villosa* Roth.) as a cover crop in subtropical regions to suppress weeds, reduce soil erosion, and increase organic matter to improve soil fertility has been proposed to enhance the sustainability of agricultural practices (Zougmore et al. 2006; Anugroho et al. 2009b). Anugroho et al. (2009b) reported that hairy vetch produced shoot biomass of 400.0 g DW m^{-2} associated with N production of 14.0 g m^{-2} in subtropical Okinawa. Weed biomass was suppressed by living hairy vetch by 62.8% during the spring fallow. In addition, Teasdale and Daughtry (1993) reported that hairy vetch living mulch suppressed weeds better than mulch formed from plant residues. Decomposition of hairy vetch following tillage during a fallow period before the subsequent crop may increase available nutrients in the soil and stimulate weed growth before the crop is sown.

The weed biomass that developed after the soil surface was covered for 1 month with dead mulch formed from hairy vetch was reported previously for subtropical Okinawa (Anugroho et al. 2009b). In addition, the effect of hairy vetch incorporation on increase grain yield of corn and P availability in soils has been documented (Astier et al. 2006; Zougmore et al. 2006). However, the effect of hairy vetch residue incorporation on weed growth and soil inorganic-N dynamics in subtropical regions has not been examined.

The objectives of the present study were to determine for a subtropical region (i) accumulations of biomass and N in hairy vetch and weeds; (ii) effects of hairy vetch living mulch and its subsequent incorporation on weed growth and soil inorganic N; and (iii) decomposition and nutrient release of hairy vetch biomass after incorporation.

2.4.2. Materials and Methods

Experimental site and soil properties

The study was conducted at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. The chemical properties of the dark red soil (Luvisols) were as follows: pH 6.1, EC 4.6 mS m^{-1}, total C 11.6 g kg^{-1}, total N 1.3 g kg^{-1}, C/N ratio 8.7, NH_4-N 5.1 mg kg^{-1}, NO_3-N 2.9 mg kg^{-1}, total P_2O_5 32.1 g kg^{-1}, and P_2O_5 absorption coefficient 11.1 g kg^{-1}.

Growing hairy vetch

Two plots of 7 m x 12 m were plowed by rotary tiller. Hairy vetch was then sown in one plot (HV+) and the other plot was left as a fallow control (HV–) in which weeds were allowed to develop without physical and chemical control. The seeds of hairy vetch (*Vicia villosa* Roth. cv. Mameko; Kaneko Seeds Company, Gunma, Japan) were manually sown on 14 November 2007 at a 0.25 m inter- and intra-row spacing, with five plants per hill and 16 hills m^{-2}. No fertilizer was applied to the soil. The plants remaining in both plots at the end of the experimental growth period were mowed and incorporated into the soil on 16 April 2008 immediately after the last harvest of hairy vetch. Thereafter the plots were left in fallow for approximately 2 months. Weeds were allowed to develop without physical and chemical control during the fallow period.

Plant and soil samplings

Weeds were counted in 0.5 m x 0.5 m by placed 10 quadrats per plot randomly on 19 December 2007. Hairy vetch and weeds were sampled by harvesting all above-ground biomass in 3 quadrats of 0.5 m x 0.5 m placed randomly on 20 February, 19 March, and 16 April in 2008 (Picture 2.4.1). Soil samples were obtained by removing the entire volume of soil from 3 quadrats of 0.5 m x 0.5 m at a 0.10-m soil depth placed randomly in 20 February and 16 April in 2008.

During the fallow period, both weed and soil samples were obtained by the same method that explained above. Weeds were sampled on 18 June (Picture 2.4.2) and 9 July in 2008. Soils were sampled on 8 May and 18 June in 2008.

Decomposition of hairy vetch

Shoots of hairy vetch were cut into 3-cm lengths and placed into 27 litterbags (10 cm x 10 cm) at a rate of 7 g DW per bag. The litterbags were buried to a depth of 0.10 m in the HV+ plot on 24 April 2008. Nine litterbags were sampled on each of the dates 22 May (28 days after buried; DAB), 19 June (56 DAB), and 17 July (84 DAB) in 2008.

Plant and soil analysis

All plant samples were dried at 70 °C for 72 h, weighed, and then ground to a powder. Total N concentration was determined using a CHN Coder (JM10; G-Science Laboratory Company, Tokyo, Japan). Soil NH_4-N and NO_3-N were determined by steam distillation after extraction with 2 mol L^{-1} KCl (Mulvaney 1996). Other soil properties were determined as reported by Anugroho et al. (2009b).

Statistical analysis

The mean values of the data were compared with Fisher's protected least significant difference (LSD) test at $P < 0.05$, where one-way analysis of variance with completely randomized block design indicated that there was a difference between means. The effects of presence and absence of hairy vetch were evaluated using Dunnet's *t*-test at $P<0.05$. Statistical analysis was carried out with the software package SPSS 14.0 for Windows (SPSS, Chicago, IL, USA).

2.4.3. Results

Biomass, N uptake and C/N ratio of hairy vetch

Shoot biomass of hairy vetch increased significantly over the period from February to April (Fig. 2.4.1A) and reached a total of 447 g m^{-2} by the end of the spring growth period in April. The shoot biomass of hairy vetch had increased 1.8 times at the flowering stage in the late April harvest compared to the vegetative stage in the February harvest.

Shoot-N uptake of hairy vetch did not significantly increase during the growing period of hairy vetch (Fig. 2.4.1B). Shoot N uptake of hairy vetch in the HV– plot ranged from 7.5 to 11.4 g m^{-2}, about three times that of weeds. The C/N ratios of hairy vetch from February to April were less than 20.

Decomposition and decline in N content of hairy vetch residue

The dry weight of buried hairy vetch residue decreased to 52% of the initial value at 28 DAB and 31% at 84 DAB (Fig. 2.4.2A). The amount of N remaining in the hairy vetch residue decreased to 51% of the initial value at 28 DAB, after which no significant decrease was found (Fig. 2.4.2B). The C/N ratio of hairy vetch residue decreased from an initial value of 18 to a value of 12 after 84 DAB (data not shown).

Weed emergence in the presence of hairy vetch

The dominant species of the weeds that established during the growing period of hairy vetch was *Bidens pilosa*. Weed emergence in December tended to be lower (*t*-test, $P \leq 0.056$) in the HV+ plots than in the HV– plots (Fig. 2.4.3A). Hairy vetch living mulch suppressed the number of weeds that emerged to about 65% of the number emerging in the absence of hairy vetch.

The shoot biomass of weeds in the HV+ plot did not change significantly between February and April (Fig. 2.4.3B). Shoot biomass of weeds in the HV– plot increased significantly from February to March and showed a slight but not statistically significant increase from March to April. After incorporation of the hairy vetch living mulch, the shoot biomass of weeds in both plots increased significantly from June to July. After incorporation, the shoot biomass of weeds was significantly greater in the HV+ plot than in the HV– plot. In the measurements during the period after incorporation, the shoot biomass of weeds in the HV+ plot was 13–29% of that which existed before incorporation and 3–6 times higher than that in the HV– plot.

Shoot N uptake and C/N ratio of weeds

Shoot-N uptake of weeds was significantly lower in the HV+ plot than in the HV– plot in February and March, but not in April (Fig. 2.4.4). After incorporation, the shoot N uptake of weeds in the HV+ plot was significantly higher than that of weeds in the HV– plot. The shoot N uptake of weeds in the HV+ plot was only 55–69% of that in the HV– plot before incorporation but 2–6 times higher than in the HV– plot after incorporation. The C/N ratios of weeds from February to April were less than 20 in the HV+ plot but 29–51 in the HV– plot. After incorporation, the C/N ratio of weeds was 19–32 in both plots.

Soil inorganic N during the living mulch stage and after incorporation of hairy vetch

Total inorganic N was calculated as the sum of NH_4-N and NO_3-N contents and ranged from 7.2 to 13.2 mg kg^{-1} in the HV+ soil during the experimental period, considerably greater than the 2.0 to 6.0 mg kg^{-1} in the HV– soil (Fig. 2.4.5). Moreover, the soil NH_4-N content was significantly higher in the HV+ plot than in the HV– plot throughout the growing period and after incorporation. The trend over time was that NH_4-N in both the HV+ and the HV– soils decreased significantly between February and April and displayed no clear change from April to June in the HV+ soil, but significantly increased from April to May and then significantly decreased in June in the HV– soil. The NO_3-N content of the HV+ soil was also significantly higher than that of the HV– soil throughout the experimental period. The trend in NO_3-N content over time in the HV+ soil was a significant increase in the period from February to April followed by a significant decrease after incorporation from April to June. In contrast, the NO_3-N content of the HV– soil decreased significantly in April and increased slightly in June.

2.4.4. Discussion

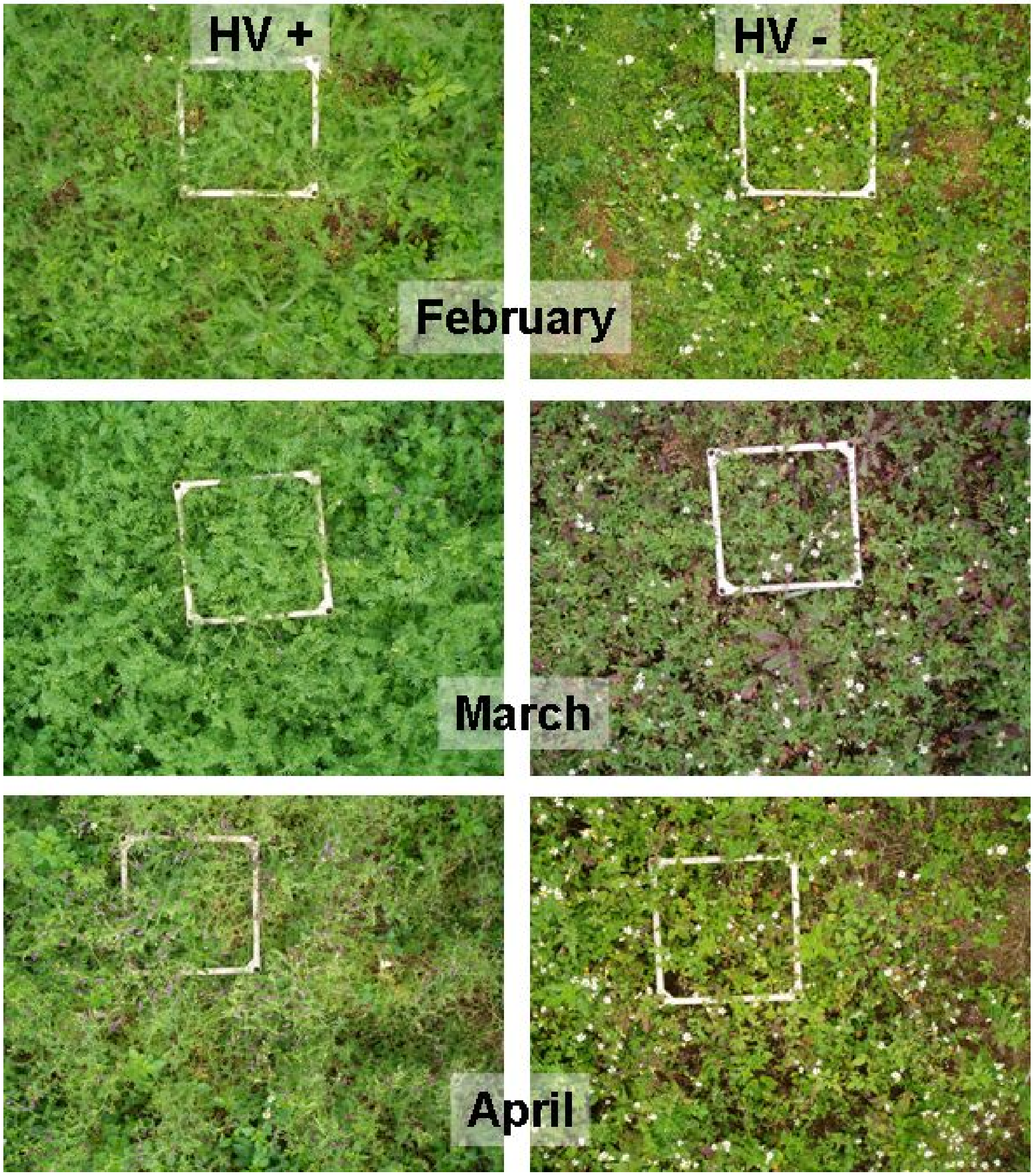
The shoot biomass of hairy vetch obtained in the present study was similar to that obtained in our previous study in Okinawa (Anugroho et al. 2009b). The inhibition of weed emergence in the HV+ plots in December compared with in the HV– plots was presumably due to competition for light, nutrients, and water by the living mulch (Teasdale and Daughtry 1993; Anugroho et al. 2009b) and possibly due to the release of phytotoxic compounds such as cyanamide, methanol, and ethyl acetate from hairy vetch (Kamo et al. 2003; Hill et al. 2007). However, after hairy vetch was incorporated into the soil, the weed biomass increase was much greater in the HV+ plot than in the HV– plot. In contrast with the living mulch stage, after incorporation weeds grew vigorously without competition for resources, and weed growth was further promoted by the elevated nutrients in the soil from the decomposition of vetch; if there was any inhibition by phytotoxic compounds after incorporation, it was obscured by the generally favorable conditions for weed growth.

The N accumulation by hairy vetch in the present study was within the range reported by Anugroho et al. (2009b). The N accumulation by hairy vetch indicated sufficient N availability to meet most of the N requirements of corn when grown under subtropical conditions (Zougmore et al. 2006). The higher soil N availability during the growing period of hairy vetch from February to April in subtropical Okinawa was also observed in a temperate region (Kuo and Jellum 2002). The fact that the C/N ratios of hairy vetch and weeds in the HV+ plot were similar and significantly lower than the C/N ratio of weeds in the HV– plot during February and April suggested that weeds grown in the presence of hairy vetch have a lower C/N ratio than weeds growing in conventional fallow soil.

Within 28 to 84 DAB, hairy vetch residue remaining was 48–69% of the original amount buried in the litter bags, and 49–58% of N in the residue had been released. The N release was similar to the 30–60% remaining within 8 weeks of burying green manure in a study by Thonnissen et al. (2000). Residue with a C/N ratio less than 20 has been reported the rapid decomposition rate of residue and nutrient release (Vigil and Kissel 1991; Kalburtji et al. 1998). In the absence of a crop after incorporation of hairy vetch, the high amount of N released into soils may have been rapidly absorbed by weeds during the spring and summer.

The decrease in NH_4-N content from February to April in the HV+ plot may have been due to N mineralization because NO_3-N content increased during this period. In contrast, the significant decrease in NO_3-N content in the HV+ soil after incorporation of hairy vetch was presumably caused by leaching because NH_4-N content remained stable.

Differences were found between the hairy vetch living mulch treatment and control on weed growth and soil inorganic-N, both before and after incorporation of living mulch. The hairy vetch living mulch effectively suppressed weeds, but weeds grew more vigorously within 84 days of hairy vetch incorporation compared with the absence of hairy vetch. The soil inorganic N content was significantly higher during the living mulch stage than after incorporation. Moreover, more than half the hairy vetch residue decomposed and more than half of the N contained in this residue was released into the soil within 2 months after incorporation. The crop that follows a living mulch stage should therefore be sown within 2 months after incorporation of the living mulch to take advantage of the nutrients released from the living mulch residue that would otherwise be lost by either runoff or leaching. Future studies should focus on the allelophaty compound derived from hairy vetch residue before and after incorporation and its effect on the emergence and establishment of weeds in subtropical regions.



Picture 2.4.1. Growth performance of field-grown hairy vetch (HV+) in comparison with control (HV-) harvested from February to April 2008.

HV +　　　　HV -

Picture 2.4.2. Weed growth in HV+ plot and HV- plot at 63 days after incorporation in June 2008.

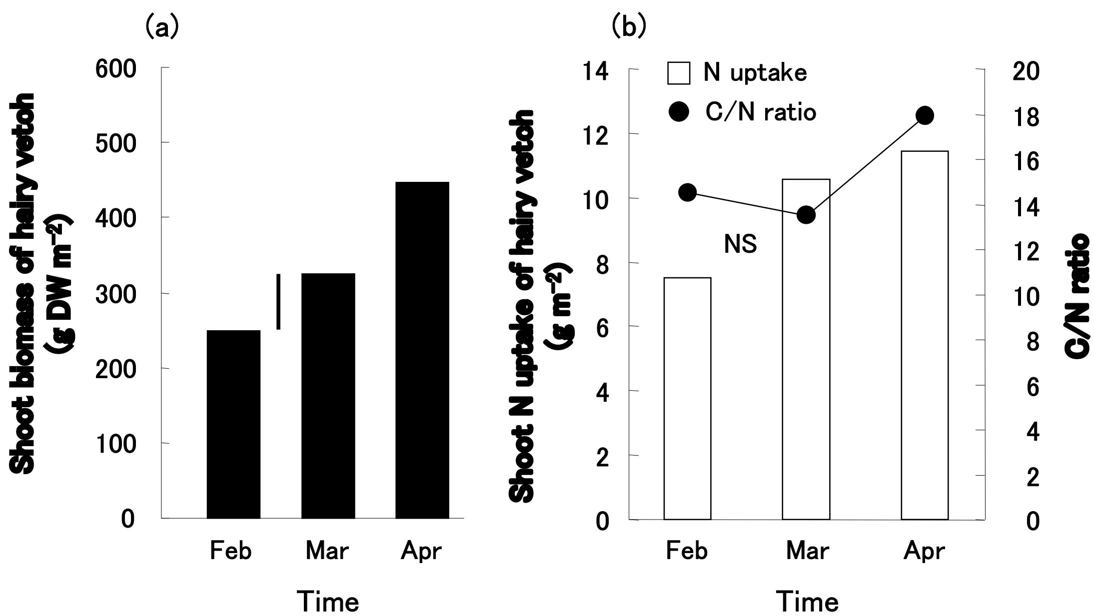


Fig. 2.4.1. Shoot biomass, N uptake and C/N ratio of hairy vetch during the growing period from February to April. Vertical bars show a significant difference, according to the Least Significant Difference test ($P < 0.05$). DW, dry weight; NS, no significant difference.

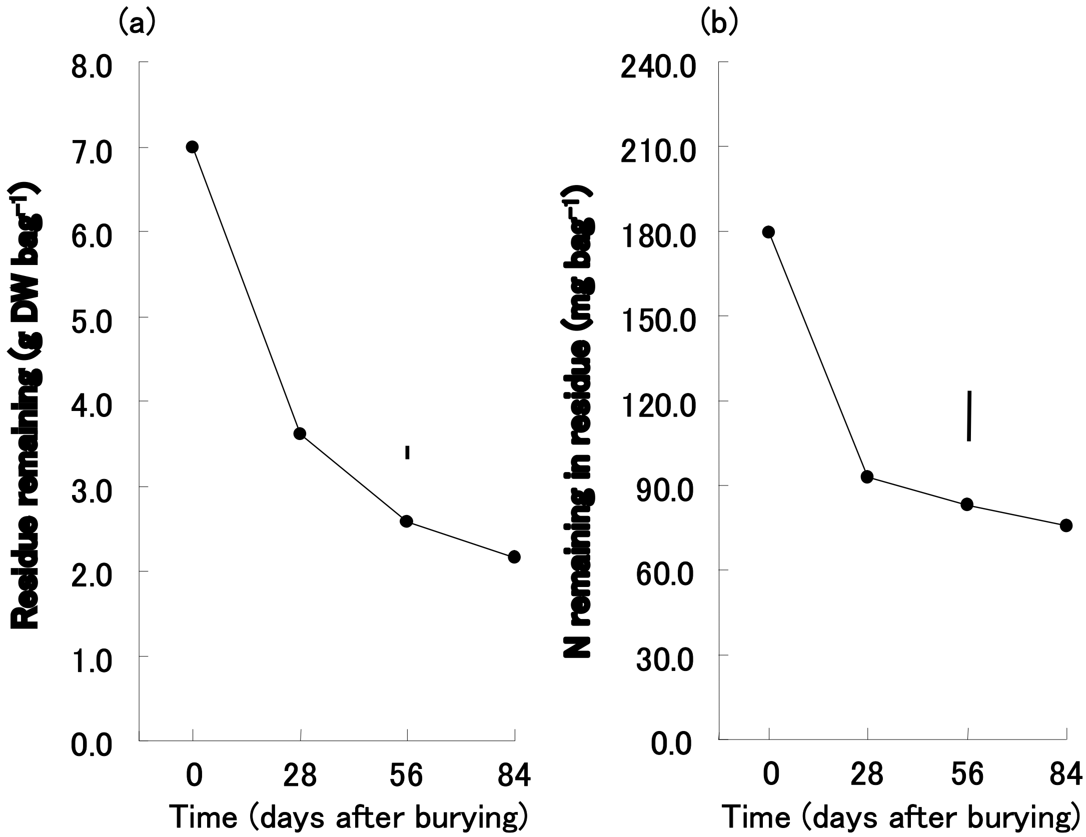


Fig. 2.4.2. Residue decomposition and N remaining in the incorporated hairy vetch residue. Vertical bars indicate a significant difference among burying times, according to the Least Significant Difference test (*P*<0.05, n = 9).

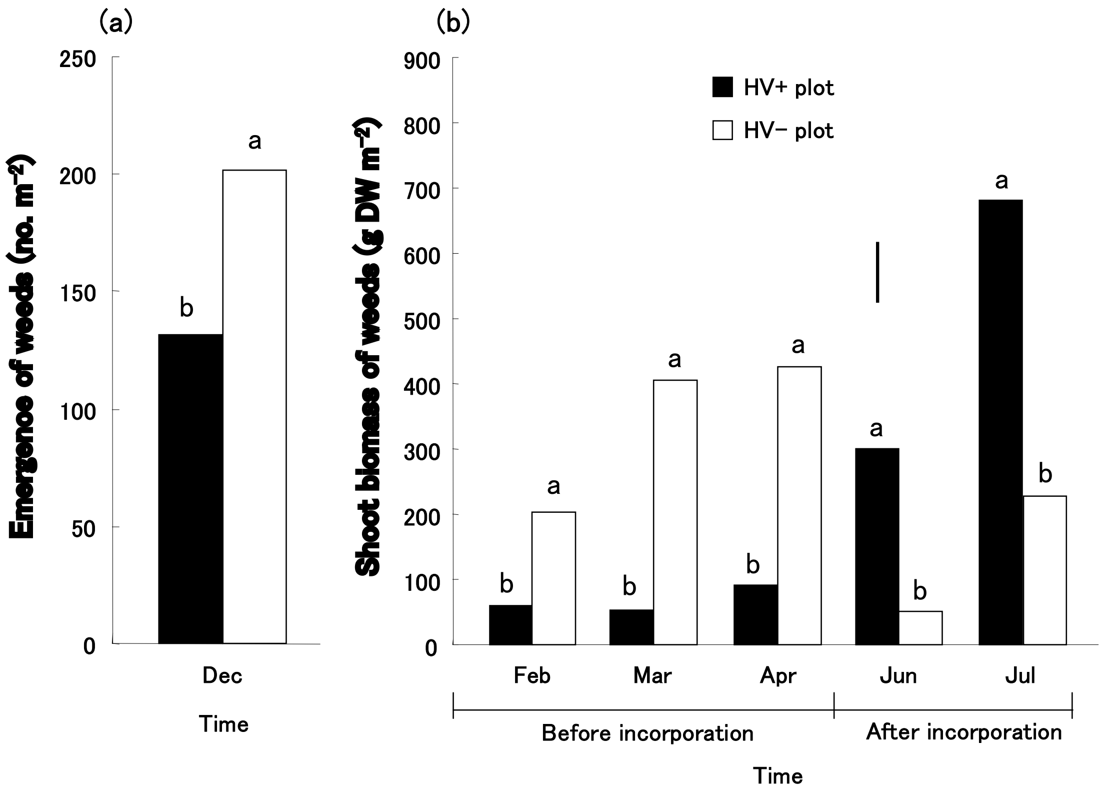


Fig. 2.4.3. Emergence (a) and shoot biomass (b) of weeds in HV+ and HV– plots during the growing period of hairy vetch living mulch and after incorporation. Vertical bars show a significant difference, according to the Least Significant Difference (LSD) test (P < 0.05, n=3). The different letters show a significant difference for each sampling date, according to the t-test (P<0.05, n = 3). no, number; DW, dry weight.

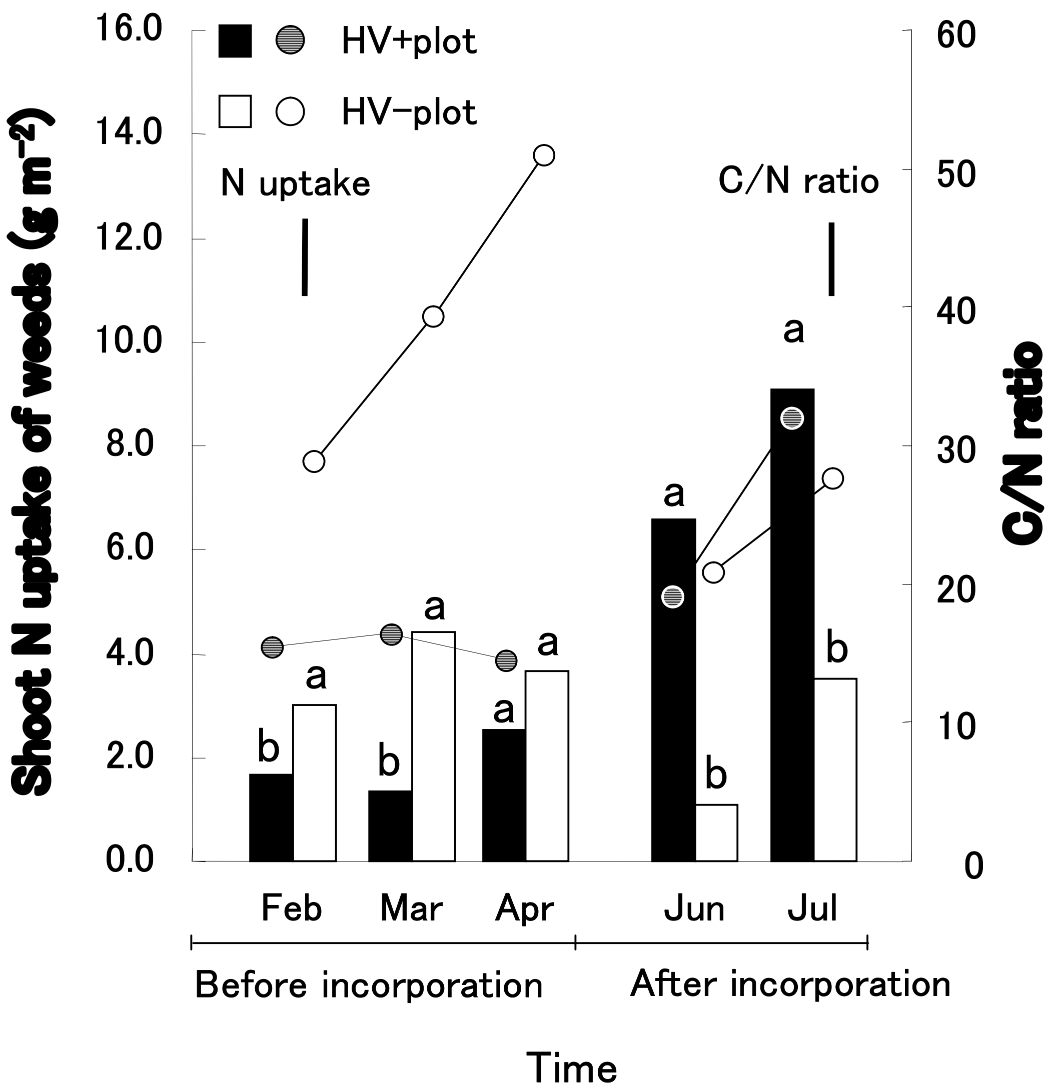


Fig. 2.4.4. Shoot N uptake and C/N ratio of weeds during the experimental period. Columns: N uptake, circles: C/N ratio. Vertical bars show a significant difference, according to the Least Significant Difference test ($P < 0.05$, n=3). The different letters show a significant difference for each sampling date, according to the *t*-test ($P<0.05$, $n = 3$).

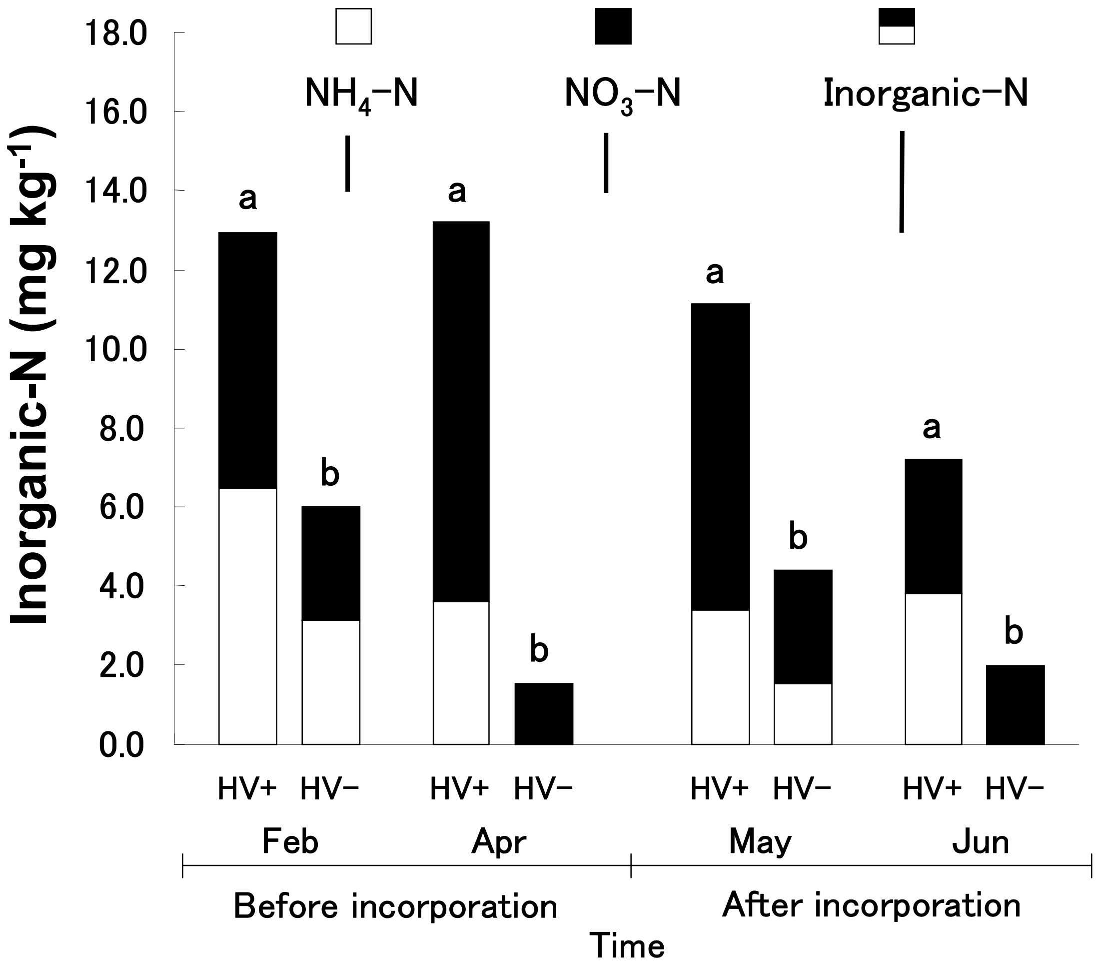


Fig. 2.4.5. Inorganic-N in soil of the hairy vetch plot (HV+) and the plot without hairy vetch (HV–). NH_4-N, Ammonium-N; NO_3-N, nitrate-N. Vertical bars show a significant difference, according to the Least Significant Difference test ($P < 0.05$, n=3). The different letters show a significant difference for each sampling date, according to the *t*-test ($P<0.05$, $n = 3$).

2.5. Effect of No-tillage and Tillage Practices on Hairy Vetch Residue, Soil Inorganic-N and Corn Growth

2.5.1. Introduction

In recent years, there has been increasing attention paid to tillage operation for crop production in tropical and subtropical regions. Tillage systems are separated into two types as conservation tillage and conventional tillage (Peigne et al. 2007). Conservation tillage included no-tillage has many advantages in crop production system (Peigne et al. 2007) and weed control management (Hiltbrunner et al. 2007). In contrast, conventional tillage may cause tons of nutrients are dissolved and transported with runoff sediments from agricultural land annually (Oyedele and Aina 1998). High decomposition rates of soil organic matter associated with degradation processes which is accelerated by tillage operation in cultivated soils present a very disturbing problem. Sanchez and Logan (1992) showed that soil organic matter in the tropics decomposes five times faster than in temperate regions.

Hairy vetch, as a preferred winter legume cover crop in subtropical regions to suppress weeds in agricultural field, has been well documented (Zougmore et al. 2006; Anugroho et al. 2009b). Moreover, the percentage of weed suppression was 63, compared with the fallow soil has been reported previously (Anugroho et al. 2009b). It was probably due to the light competition (Teasdale, 1993) and the allelopathic compounds (Teasdale and Daughtry 1993; Hill et al. 2007). Biological N fixation (BNF) reduced the application of N fertilizers for subsequent crops (McVay et al. 1989; Clark et al. 1995; Kuo and Jellum 2002). Furthermore, N supply by shoot and root of hairy vetch through tillage and no-tillage systems may increase inorganic-N level in soils and contribute to better agronomic practices for sustainable agriculture. The advantages of including hairy vetch as cover crops into corn and sorghum production systems are well documented for the subtropical and temperate region of Japan (Zougmore at al. 2006; Choi and Daimon (2008); however, the distribution of the ammonium-N and nitrate-N in the soil-depths and the effect on the growth of shoot and root of corn is not well understood.

The objectives of this study were to observe the biomass and nutrient accumulation of shoot and root of hairy vetch which is applied as green manure for the growth of subsequent corn. Another attempt is to determine ammonium and nitrate levels of different soil-depths after hairy vetch addition and their effects on corn growth.

2.5.2. Materials and Methods

Experimental site and soil chemical properties

Experiment was conducted at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan (26°N, 127°E). The soil type was red soil. pH and electrical conductivity of the initial soil were 5.0 and 9.8 $x10^{-3}$ S m^{-1}, respectively. Total C, total N, C/N ratio, inorganic N and phosphate absorption coefficients were 1.3 g kg^{-1}, 0.3 g kg^{-1}, 5.1, 3.1 mg kg^{-1}, 4.1 g kg^{-1}, respectively.

Plant materials and experimental design

A 1/5000 a Wagner pot was filled with a mixed soil of 4.0 kg of red soil ("Kunigami Mahji") and fertilizers of 1.0 g N (urea), 0.5 g P_2O_5 (superphosphate) and 0.5g K_2O (potassium sulfate). Initially, two treatments were provided, one was grown hairy vetch and another was without hairy vetch. Five seeds of hairy vetch (*Vicia villosa* Roth cv. Mameko, Kaneko Syubyo) were sown on 20 November 2008. Plants were thinned to two plants per pot at 14 DAS. Plants were grown in outdoor and then harvested on 3 March 2009 (Picture 2.5.1). All shoots of hairy vetch were sampled. Because roots of hairy vetch were used for input of subsequent corn, only four pots were sampled for nutrient analysis. The shoot and roots were dried at 70°C for 72 h, weighed. Prior to chemical analysis, the dried sampled was powdered. On the other hand, for shoot addition to subsequent corn growth, the dried shoots were cut in 2-3 cm length.

Prior to planting corn, the fertilizers with rate of 1.0 g N, 0.5 g P_2O_5 and 0.5g K_2O were applied together with preparing six treatments below. Fourteen gram of the dried shoots and fertilizer were applied and tilled with the soil containing roots (SRT) and the other one was put them on the surface of the soil containing roots as mulch or no-tillage (SRN). Only fertilizers were applied and tilled (RT) and no-tillage (RN) in the pots containing roots. Similarly, pots without hairy vetch addition were treated by tillage (CT) and no-tillage (CN) as control. There were two pot-conditions. One was planted-corn pots for plant growth measurement and another was without corn for determining soil chemical properties. Soils were tilled on 10 March 2009. Three seeds of Corn (*Zea mays* L.) were sown in each pot on 7 April 2009. Shoots and roots of corn were harvested on 2 June 2009, as shown in Picture 2.5.2 and Picture 2.5.3, respectively. Soils were sampled at three soil-depths (0-5 cm, 5-10 cm and more than 10 cm) and three times on 19 March (9 days after tillage/DAT), 2 April (23 DAT) and 12 May (65 DAT). The measurements were performed with three replications.

Plant and soil analysis

The dried shoot and root of plants were powdered prior to analysis. Total C and N concentrations were determined by CHN Coder (JM10, G-Science, Lab. Co., Ltd., Japan). Total P concentration was determined by calorimetric methods after drying ash at 450°C for 1h and dissolving it in 1:30 (v/v) diluted nitric acid (Hafner et al., 1993). The concentrations of K, Ca and Mg of all samples were determined using an atomic absorption spectrophotometer (AAS) (Solaar 969; Japan Thermo Corp., Tokyo, Japan) after Kjeldahl degradation.

The soils were air-dried and passed through a 2 mm sieve. The soil pH and EC were measured in water at 1:2.5 and 1:5 (w/v), respectively. The total C and N concentrations were determined using a CHN coder (JM10; G-Science Laboratory Company, Tokyo, Japan). The inorganic nitrogen was determined by steam distillation after extraction with 2 mol L^{-1} KCl (Mulvaney 1996). Ca-bound phosphate, as the available P_2O_5, was determined by calorimetric methods after extraction with 0.025 L L^{-1} of acetic acid. The phosphate absorption coefficient was determined by calorimetric methods after absorption with ammonium hydrogen phosphate. The exchangeable cations were determined by AAS after extraction with ammonium acetate.

Statistical analysis

The data are the mean values of three replicates with the standard error of the mean. The mean values of the data were compared by one-way analysis of variance (ANOVA) with a completely randomize design. The mean comparisons were evaluated using a Fisher's protected least significant difference (LSD) test at $P<0.05$. Statistical procedures were carried out with the software package SPSS 14.0 for windows.

2.5.3. Results

Biomass addition and nutrient supply by hairy vetch

Biomass of shoot and root of hairy vetch harvested at 103 days after sowing (DAS) was 23.3 and 10.0 g DW pot^{-1}, respectively (Table 2.5.1). However, only 14 g DW pot^{-1} of shoot biomass was added into pots due to the limited upper-space of pots. Total biomass addition of hairy vetch was 24 g DW pot^{-1} may supply some nutrients by releasing them through decomposition. The total biomass addition of hairy vetch might be associated with the nutrients input (mg pot^{-1}) of 955.0 N, 77.5 P, 147.6 K, 1957.6 Ca and 78.5 Mg. In addition, the nutrients for corn growth were not only derived from decomposing of hairy vetch, but also from fertilizers that applied at the same time. They were taken up by corn during its vegetative growth.

Ammonium-N and nitrate-N levels distribution

The ammonium-N (NH_4-N) content in the upper (0-5 cm depth), middle (5-10 cm depth) and deeper layers (>10 cm depth) of the soils for no-tillage and tillage treatments showed was decreased during the growing period of corn (Fig. 2.5.1A). In the upper layer (0-5 cm depth) of the soils, the NH_4-N content at 9 and 23 days after treatment (DAT) for no-tillage was higher than for tillage. The NH_4-N content in the upper layer of the soils showed higher for the shoot and root additions of tillage than for root addition and control of tillage. In the middle and deeper layers of the soils, the NH_4-N content of the soils at 9 DAT was lower for no-tillage than for tillage.

The nitrate-N (NO_3-N) in all layers of the soils for no-tillage and tillage treatments was increased during the growing period of corn (Fig. 2.5.1B). The NO_3-N content in all layers of the soil at 9 DAT showed a similar content and higher in the control of tillage (CT), but it was lower at 23 and 65 DAT for no-tillage than for tillage. The NO_3-N content in the upper layer of the soils showed lower for the shoot and root additions of tillage than for root addition and control of tillage.

Plant height, shoot and root biomass of corn

During the growing period of corn, N was supplied from the N fertilizer and hairy vetch residue in no tillage and tillage. Plant height of corn during 14 to 56 DAS under SRN and RN treatments had a tendency to be higher than under CN treatment in no-tillage and higher than SRT, RT and CT treatments in tillage system (Fig. 2.5.2). In addition, the presence of hairy vetch residues in no-tillage showed the wider stem diameters of corn from the first growing period and more light-green leaves during 42-56 DAS than that in tillage. The plant height of corn under CN showed a similar level with addition of hairy vetch in tillage and tended to be higher than CT. However, the plant height of corn treated with hairy vetch in no-tillage and tillage was relatively higher than that in control.

The shoot and root biomass of corn under no-tillage was higher than tillage (Fig. 2.5.3). Furthermore, the shoot biomass of corn under SRN was tended to be higher than RN. Similarly, it was higher under RN than CN in no-tillage. Meanwhile, the shoot biomass of corn under SRT was higher than RN and RT. In addition, the shoot biomass of corn under CN was relatively higher than that under CT. Root biomass of corn at three soil-depths had a similar pattern with shoot biomass. The distribution of the root biomass was higher in the upper and the deeper layers than in the middle layer.

2.5.4. Discussion

In the present pot experiment, hairy vetch grown under red soil was flowered in February, similar with the previous study under Akadama soil. (Anugroho et al., 2009), but earlier compared with the data reported in temperate region (Choi and Daimon, 2008). The biomass dry weight of shoots and roots of hairy vetch was obtained higher than that of hairy vetch reported by Choi and Daimon (2008). Percentages of hairy vetch biomass additions by the shoots 60 and the roots 100, compared with their initial biomass, were input as green manure into soils under no-tillage and tillage together with secondary fertilizer applications for the vegetative growth of corn. Seo et al. (2006) reported that the combination of N from fertilizer and cover crop into an integrated organic-N and inorganic-N management system could realize the positive benefits of each source in corn production; the percentage of N recovery of hairy vetch residues and N fertilizer in the soil was 55 and 27, respectively. The fertilizer may satisfy to supply the major nutritional needs of the crop, and the cover crop residues contributing more N for maintaining soil N resources.

The additional biomass of the shoots and roots in no-tillage and tillage affected the ammonium-N and nitrate-N contents in the soil profiles. The ammonium content of soils was declined and converted to the nitrate-N content during 9 to 65 DAT in no-tillage and tillage. It was presumably due to the mineralization was higher rather than immobilization. The high mineralization was occurred in the tillage rather than in no-tillage. Sainju et al (2006) noted that tillage influences the mineralization of cover crop residue and the soil N level. The ammonium-N content was highly concentrated in the upper layer for no-tillage, in contrast with tillage at 9 DAT. It was probably due to the different distribution of the ammonium-N content, where hairy vetch residue and N fertilizer were initially placed at the upper layer in no-tillage, in contrast with tillage. In addition, the release of mineralized N following degradation of tilled cover crop residue depends on a variety of factors, including quantity of residue, tissue N concentration or C/N ratio (Kuo and Jellum 2002). Generally, the nitrate-N content of the soil layers in no-tillage was lower than in tillage during 9 to 65 DAT. It may indicate that the release of the nitrate-N content in the no-tillage was slower than that in tillage.

Corn height was highly affected by the shoots and roots of hairy vetch addition in no-tillage rather than that in tillage. Corn height was increased during 14 to 42 DAS related to the increase of the nitrate-N content of the soils during 23 to 65 DAT. Similarly, the biomass dry weight of shoots and roots of corn was higher in the no-tillage than that in tillage. This is consistent with the previous study reported by Zougmore et al. (2006); the corn growth was higher when cultivated in the presence of hairy vetch with zero tillage than tillage and natural fallow.

Under no-tillage, the shoot and root growth of corn was significantly higher for the shoots and roots addition than for the absence of hairy vetch and tillage treatments. Even though the hairy vetch residue of no-tillage that was decomposed slower and slowly released nutrient into the soils than that of tillage, the high NH_4-N and NO_3-N contents for tillage might be lost by leaching and denitrification more rapidly than for no-tillage. Therefore, the high nitrate-N release in tillage may able to leach into the ground and more serious problem rather than no-tillage. Seo et al. (2006) reported that the N loss percentage was varied in the range of 42-48 through leaching and denitrification. Further field study is needed to evaluate the effects of hairy vetch addition and no-tillage on soil-N availability and yield production of corn.

Picture 2.5.1. Performance of pot trial hairy vetch in March 2009 before incorporation/mulch and planting subsequent corn.

Table 2.5.1. Nutrient input by additions of hairy vetch and fertilizer prior to planting corn.

	Shoot	Root	Fertilizer
Biomass harvest (g DW pot^{-1})	23.3	10.0	
Biomass Input (g DW pot^{-1})	14.0	10.0	
Nutrient input (mg pot^{-1}):			
Nitrogen (N)	591.4	363.6	1000.0
Phosphorus (P)	45.5	32.1	109.1
Potassium (K)	105.4	42.2	207.5
Calcium (Ca)	1352.9	604.7	
Magnesium (Mg)	25.3	53.2	

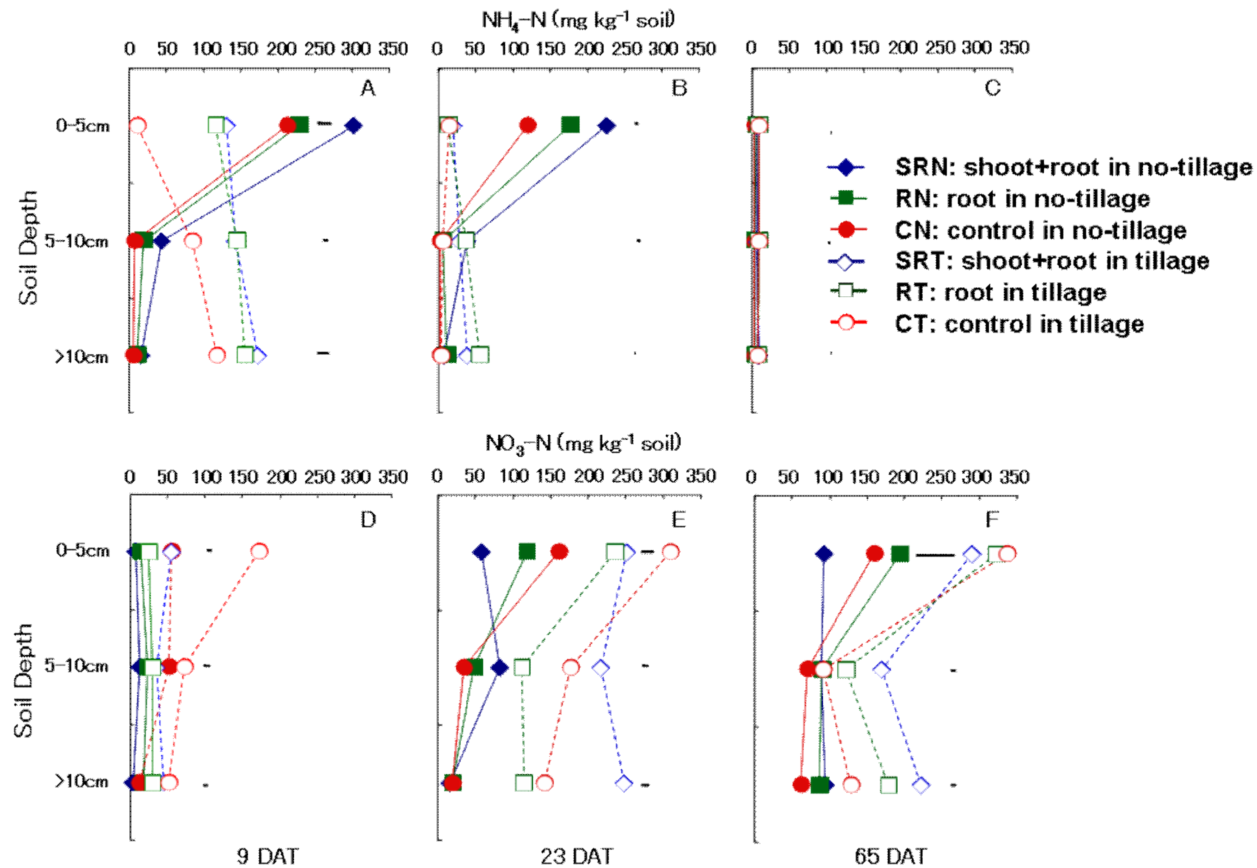


Fig. 2.5.1. Ammonium-N and nitrate-N levels distributed in three soil-depths (0-5 cm, 5-10 cm and >10 cm) at 9, 23 and 65 days after tillage (in absent of corn). Shoot and root addition (SRN and SRT), root addition (RN and RT) and without hairy vetch addition (CN and CT) under no tillage and tillage conditions. Vertical bars represent LSD ($p<0.05$.

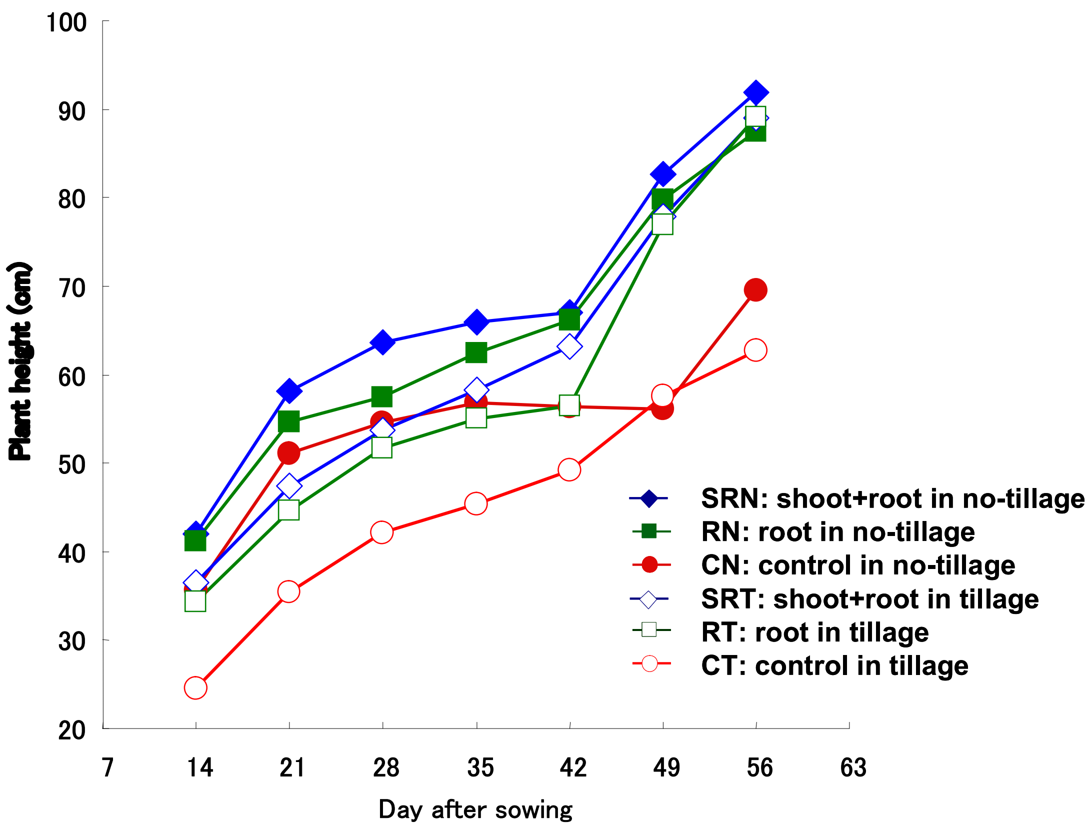
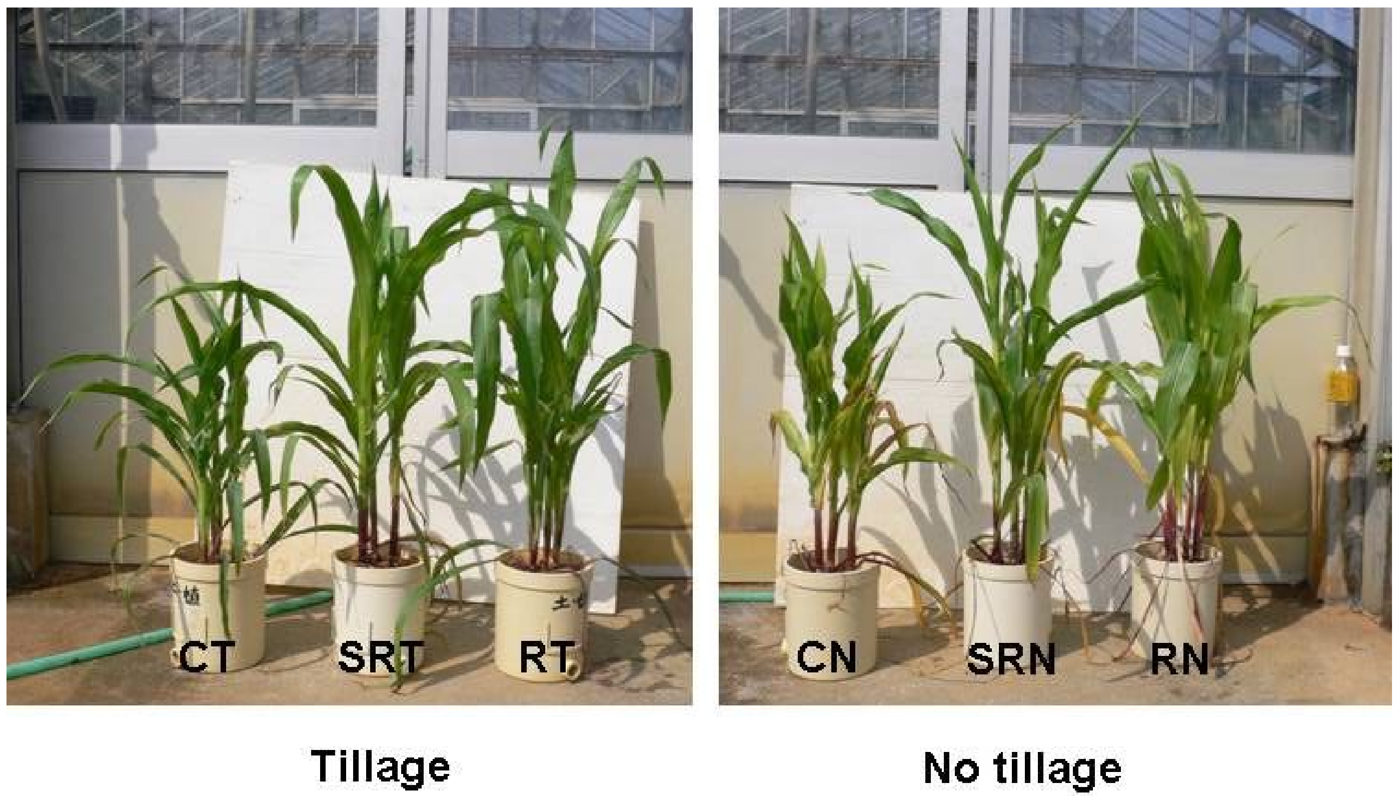


Fig. 2.5.2. Plant height of corn during the growing period for 56 days after sowing.



Picture 2.5.2. Top growth of corn under application of the shoots and root (SR), roots (R) and control (C) in tillage and no tillage cropping systems at 56 days after sowing.

Picture 2.5.3. Root growth of Corn under application of the shoots and root (SR), roots (R) and control (C) in tillage and no tillage cropping systems at 56 days after sowing.

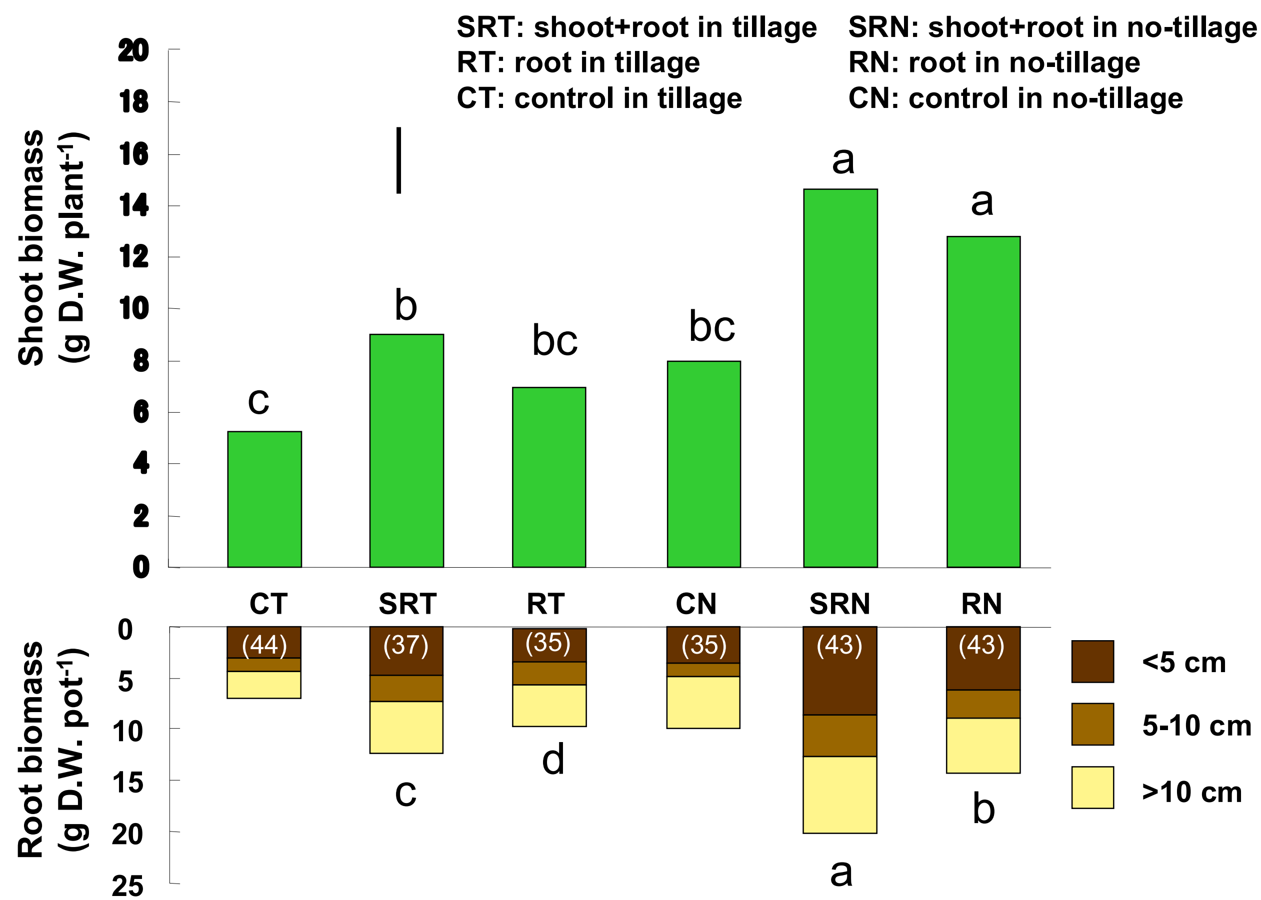


Fig. 2.5.3. Biomass of shoot and root of corn harvested at 56 days after sowing. The different numbers show a significant difference. The vertical bar represent a significant difference, according to the Least Significant Difference at $P < 0.05$ ($n = 3$). The numbers in the parentheses were the percentages of the root biomass ratio of the total root biomass

Chapter 3

General Discussion

The acid-weathered soils of the tropical and subtropical regions are particularly prone to phosphorus (P) deficiency and aluminum (Al) toxicity (von Uexkull and Mutert, 1995). P is unavailable because it rapidly forms insoluble complexes with cations and is incorporated into organic matter by microbes. The acid soils with high levels of iron (Fe) and aluminum (Al) have high phosphorus (P) sorption capacity leading to low P availability to plants (Jemo et al 2006). Von Uexkull and Mutert (1995) recommended that the first phase of reclaiming acid soils, particularly low in N and P, is the use of a legume cover crop supported by liming and conservative P application.

Integration of the winter legume cover crop in a cropping system results higher soil organic matter content, increased nutrient availability for subsequent crops, and effective weed suppression in temperate region. Many studies have indicated less weed suppression in cropping systems with plant residues than in systems with living mulch (Ilnicki and Enache, 1992; Teasdale and Daughtry, 1993). Depending upon the management and cropping system, legume green manures have the potential to replace more than 100 kg N ha^{-1} for a subsequent grain crop (Zougmore et al. 2006). Not only would a savings in N fertilizer occur with expanded use of legumes in intensive agriculture, but also the potential for N leaching into groundwater and volatilization of N into the atmosphere could be reduced because legume N is less susceptible to the chemical and physical conversions that lead to such losses. In addition, legume cover crops can affect soil water relations especially in the spring by increasing infiltration (McVay et al., 1989) and/or reducing evaporation (Villalobos and Fereres, 1990), and by transpirational water use (Zachariassen and Power, 1991). Soil water conservation by killed cover crop residues may result in more favourable soil moisture conditions for the subsequent crop (Moschler et al., 1967; Gallaher, 1977; Corak et al., 1991; Munawar et al., 1990; Holderbaum et al., 1990; Myers and Wagger, 1991). However, a serious problem in living mulch cropping systems is yield loss of a crop because of competition. Different ways have been suggested to overcome this problem in such the planting and killing dates and cropping systems (Teasdale and Daughtry 1993; Teasdale et al. 2004).

In the pot experiments, hairy vetch might be categorized as a superior legume cover crop, and followed by crimson clover, under P-deficient soils and a short growth period, with higher shoot biomass and N-fixing activity, than several vetch and clover species. The biomass percentages of the shoots and roots of hairy vetch were 66 and 99, respectively, relatively higher than those of clover and vetch species. Under P-sufficient soils, the biomass of the shoot and roots was generally higher than that of clover species. In addition, pink clover may be an alternative legume cover crop with high C accumulation among clover species. The biomass of hairy vetch during February and April was varied ranging from 6.0 to 85.2 g pot^{-1} for the shoot and from 2.6 to 31.6 g pot^{-1} for the roots; depend on the plant cultivar, sowing date, temperature and soil type. For example, hairy vetch cultivar Common has longer growing period and higher biomass than cultivar Mametsuke. Moreover, hairy vetch is better sown during October and November resulted higher biomass in March and April, related to the average temperature during the early growing period was higher than 15^{o}C (Japan Meteorology Agency, 2009). Hairy vetch sown in the period of October-November and harvested February-April in a subtropical region was reached the shoot biomass averages of 4000 kg ha^{-1}. The shoot biomass of hairy vetch sown in October and December when harvested in February, March and April showed a significant difference. The 2 months delay in sowing date in subtropical Okinawa, the biomass reduction percentages were 30-60, of the total biomass of hairy vetch harvested during February-April. The biomass reduction due to the delay in sowing date occurred in subtropical Okinawa was higher than previously reported in temperate regions by Teasdale et al. (2004).

Weeds, dominated by *Biden pillosa*, were effectively suppressed 7 by the percentage of 71-87 through hairy vetch living mulch in the growing period of February-April, and by the percentage of 37-65 at the desiccated stage in May, in comparison with the fallow soil. Similarly, Teasdale and Daughtry (1993) reported that a living mulch of hairy vetch more effectively suppressed weeds than at its desiccated stage. However, the incorporation of hairy vetch stimulated the weed growth by 3-6 times during 63 and 84 days after incorporation. It may due to the favorable field conditions for weed growth after soil incorporation such as light and nutrients.

Generally, the nutrient accumulation of hairy vetch showed a similar pattern with its biomass. Moreover, the shoot-N production of hairy vetch was usually high during February-March harvest when plant sown in October, and during March-April harvest when sown in November. The N production of hairy vetch was approximately 3 g pot^{-1} from pot experiment and equivalent to 150 kg ha^{-1} from field experiment. The N production level of hairy vetch is sufficient to meet most of the N requirement of the high-requiring N crops such as corn and tomato (Abdul-Baki and Teasdale; Zougmore et al. 2006). The C/N ratios of hairy vetch were relatively lower than that of clover species. The C/N ratios of hairy vetch in the present study, usually lower than 20, were considerable influence on the rate of residue decomposition and nutrient release (Kalburtji et al. 1998; Vigil and Kissel 1991). With lower C/N ratios, hairy vetch was rapidly decomposed within 28-84 days after tillage, and associated with more than a half of the initial N residue released into soils. In addition, the P, K, Ca and Mg uptakes of hairy vetch during February to April were less than those levels of 15, 100, 30 and 10 kg ha^{-1}, respectively.

Even though hairy vetch absorbed N, mostly from nitrate-N, for its vegetative and flowering stages from February and April, the nitrate-N content increased during the time, and subsequently increased at the desiccated stage in May. Similarly, the available-N was noticeably higher in soils amended with hairy vetch sown earlier in October and November than that in absence of hairy vetch. It was presumably due to high N_2-fixation during the growing period. When soil left bare for 2 months after incorporation in June, the nitrate-N content was decreased, and increase again in August and October. It was probably due to the nitrate-N content derived from mineralization of hairy vetch residue decomposition.

The ammonium-N and nitrate-N content were distributed in three layers of soils, without planting corn, showed the behavior of inorganic-N during corn growth under hairy vetch addition and control in both no-tillage and tillage. In tillage, the ammonium-N content was relatively concentrated in the deeper layer at 9 days after tillage. On the other hand, in no-tillage, the ammonium-N content at the upper layer was markedly higher during 9-23 days after tillage, but lower at both the middle and deeper layers at 9 days after tillage than that in tillage. At 23 days after tillage, the additions of the shoots and roots together and the roots alone of hairy vetch at the upper layer in no-tillage found markedly higher ammonium-N content than without addition of hairy vetch. Most of the ammonium-N content was distributed in the upper layer; it was probably due to the N fertilizer was initially applied in the soil surfaces. Meanwhile, the nitrate-N content of soils in no-tillage was markedly lower than in tillage in the period of 23-65 days after tillage, where the nitrate-N content was concentrated at the upper layer in no-tillage and tillage.

The integration of hairy vetch, by increasing the soil inorganic-N content mostly in the nitrate-N form, may significantly contribute to plant growth in corn production system. The addition of hairy vetch in no-tillage showed wider stem diameters of corn from the first growing period and more light-green leaves during 42-56 days after sowing than that in tillage. The different leaf color was presumably due to the nitrate-N content in no-tillage was lower than that in tillage. The shoot biomass of corn harvested at 56 days after sowing showed markedly higher in no-tillage than in tillage culture. Similarly, the root biomass of corn in three layers was totally higher in no-tillage than in tillage. The distribution of root biomass of corn was higher in the upper and deeper layers than in middle layer.

Hairy vetch was selected as an appropriate winter legume cover crop with higher contribution of organic matter, nutrient accumulation and weed suppression in the agriculture soils in a subtropical region. Hairy vetch is a better green manure legume among winter legume cover crops under P-deficient and P-sufficient condition either in short or long growing periods. In addition, the biomass of crimson clover showed higher tolerance of P-deficient condition in a short growing period, but lower biomass in a long growing period; in contrast with pink clover. Hairy vetch can grow under subtropical soil conditions within pH of 5.7-7.5, resulting high biomass and nutrient accumulation. The nitrate-N content was increased under living mulch and dead during February to May. The release of nitrate-N content in no-tillage was slower than tillage system. In soil amended with hairy vetch residue, the corn growth showed higher in no-tillage than tillage. The living mulch of hairy vetch showed better weed suppression than the dead mulch of hairy vetch and fallow soils, but the weed growth was 3-6 times higher than fallow soils after incorporation. Therefore, the utilization of hairy vetch either no-tillage or tillage systems in the agricultural practices should be integrated with the nutrient demand of the subsequent crop in subtropical regions.

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