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Nitrogen Fixation and Utilization for Green Manure of Common Wild Legume Narrowleaf Vetch (*Vicia angustifolia* L.)

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Abstract

Narrowleaf vetch (*Vicia angustifolia* L.) (NLV) is a legume widely distributed in the Eurasian continent. Although commonly growing as a weed in cultivated areas, NLV could become a suitable species for cultivation as a green manure crop to increase the nitrogen content of soil. We examined NLV and a common green manure crop, Chinese milk vetch (*Astragalus sinicus* L.) (CMV), both in monocultures and in mixed cropping of the 2 species. The nitrogen-fixing activity and root nodule activity of the species were determined and the effects on the nitrogen (N) supply to soil were evaluated. CMV produced more plant N than NLV, largely as a response to better growth after the over-wintering period. The amount of atmospheric N fixed by NLV (4.3 g m^{-2}) was only half of that fixed by CMV (8.6 g m^{-2}). Nevertheless, the amount of N in NLV (up to 14 g m^{-2}) was sufficient to supply the demand of the following crop. The mixed culture of CMV and NLV performed better than the monoculture. Since developing countries cannot afford to invest money on nitrogenous fertilizers or legume seeds, the use of the widely distributed NLV as a green manure might be effective and contribute to the sustainability of cropping systems.

Discipline: Agricultural facilities / Soils, fertilizers and plant nutrition
Additional key words: Chinese milk vetch (*Astragalus sinicus* L.)

Introduction

In agro-ecosystems, 80% of biologically fixed N_2 may be derived from symbiosis involving leguminous plant species with *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium* and *Allorhizobium*[15]. The inclusion of leguminous crops into cereal farming systems improves their long-term sustainability, primarily because legumes fix atmospheric N but also by the addition of diversity to the agricultural systems[2,6]. Legumes can be grown in rotation with one or more rice crops per year and the nutrients in the legume crop residues are beneficial to the following rice crop[4,16]. Legumes in rotation with crops can also increase the content of soil organic matter[14]. Cereals intercropped with legumes generally benefit from the association in terms of enhanced grain and nitrogen (N) yields per unit area compared with mono-cropped cereals[1].

Legumes are usually grown as dry season crops in subtropical and temperate areas, especially, China, Japan and Korea, where the rice-growing season is restricted by low temperature during the winter seasons[3,14,16]. Chinese milk vetch (*Astragalus sinicus* L.) (CMV) is a cultivated leguminous plant, common in drained fields and on levees. Chinese milk vetch has been widely cultivated in China, Japan and Korea. The cultivation area in Japan covered nearly 220,000 and 20,000 ha in 1960 and 1989,

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respectively[16]. In contrast, there has been no attempt to cultivate narrowleaf vetch (NLV) as a legume crop in agricultural fields despite the fact that NLV commonly grows as a weed in fields and is distributed over western Asia, Southeast and East Asia. Each year, NLV naturally regenerates in upland fields from self-sown seed. The purpose of this experiment was to examine the potential of NLV as a legume green manure crop either in comparison or in combination with CMV.

Materials and methods

1. Site characterization

An experiment was conducted in 2000 at the Ehime University Experimental Farm, Japan. The site (33°57'N, 132°47'E, 15 m above sea level) was located in the temperate zone with hot humid summer and cold dry winter.

2. Crop cultures, treatments and experimental design

Three legume-rice cropping systems were maintained over a period of 4 years starting from 1997. The legume green manure crops were grown in winter (November to May) and the paddy rice crops from June to October or early November. The legume crops consisted of either monocultures of Chinese milk vetch (*Astragalus sinicus* L.) (CMV) or narrowleaf vetch (*Vicia angustifolia* L.) (NLV) or a mixed culture of the 2 species. CMV and NLV were sown in the paddy field by broadcasting 1,000 seeds m^{-2} (3.5 g of CMV and 16 g of NLV) on October 20 in each year. The mixed culture of CMV/NLV was sown with 500 seeds m^{-2} of each species. Both vetches grew well before and after winter. In the control fallow plot established from February 15 to April 28 in 1999, no legume seed was sown and weeds were removed periodically by hand. The experiment was laid out in a randomized block design in a field (200 m^2) with 4 treatments and 5 replications, with each plot measuring 6 m^2.

3. Determination of plant, soil characteristics and nitrogen-fixing activity

The 10 legume plants were harvested between 12 and 2 pm on sunny days during the period from over-wintering to ripening in each plot. Plant height and root length were measured. The plants were separated into root and shoot parts, which were dried at 75ºC for 2 days. Nodule size and weight were calculated from the 10 plants. Plant and weed densities were determined from 0.5 m^2 plots in the same area. The amount of total dry matter of plant was determined at the time of harvest by removing 10 plants from 0.5 m^2 in each plot.

Nitrogen-fixing activity of the nodulated roots and nodules was determined within 5 min after harvest. The shoot portion was cut off at the base of the plants and the soil particles attached to the roots were removed carefully by hand. The nitrogen-fixing activity represented by the nitrogenase activity (NA) was measured using the acetylene reduction activity (ARA) method with 5 replications per treatment[5]. Nodulated roots were placed in 1-L polypropylene Mason jars and sealed with rubber stoppers. Then, 100 mL of air was removed using a 50 mL syringe and replaced with 100 mL of acetylene. The Mason jars were placed in the holes from which the plants had been removed, covered with soil, and incubated at soil temperature for one hour. At the end of the incubation period, the jars were removed from the soil and, after proper mixing, 1 mL samples were withdrawn for the analysis of ethylene concentration. Samples were analyzed within 4 h by injecting 1 mL of gas into a gas chromatograph with GC condition, temperature, carrier gas speed and column type being similar to those described by Hosoda et al.[9].

After sampling of the plants in each plot, soil samples were collected from the 0 to 20 cm depth. After removal of large visible pieces of plant materials, the soil was dried and ground to < 2 mm for the determination of the total N and C contents by the combustion method using a Sumigraph C/N analyzer (NC-90A). Soil moisture content (%) was determined in 0–10 cm sub-soil by

Table 1. Changes in soil N concentration (%) in mono- or mixed cultures of Chinese milk vetch (CMV) and narrowleaf vetch (NLV) grown in a paddy field

Treatments	Date						
	Feb. 25	Mar. 2	Mar. 15	Mar. 21	Mar. 31	Apr. 7	Apr. 26
Control	0.18	0.18	0.18	0.19	0.18	0.17	0.17
CMV-mono	0.18	0.17	0.17	0.16	0.16	0.15	0.14
NLV-mono	0.17	0.16	0.15	0.15	0.15	0.15	0.14
CMV/NLV-mixed	0.16	0.15	0.15	0.14	0.14	0.14	0.14
LSD 0.05	0.012	0.011	0.011	0.013	0.022	0.021	0.021

the gravimetric method with oven drying. Soil samples were collected using soil cores 50 mm in diameter down to a 10 cm depth. Samples were weighed, oven-dried at 105ºC for 24 h, reweighed, and the mass of water lost as a percentage of the mass of the dried soil was calculated.

Results

1. Soil nitrogen concentration

The level of soil N remained constant in the fallow treatment throughout the experiment (Table 1). In contrast, legume cropping gradually reduced the soil N concentration, particularly in the Chinese milk vetch (CMV)/ narrowleaf vetch (NLV) mixed culture. The reduction in the soil N concentration was considerable during the legume-ripening period and occurred similarly in all the legume treatments.

2. Plant density, weed density and characteristics of the shoots, roots and root nodules

The density of both vetch species decreased from soon after over-wintering (Feb. 25) until ripening (April 26) in all the treatments (Fig. 1a). A larger number of CMV than NLV plants survived the over-wintering period and the relative plant densities were maintained throughout the growth period in both mono- and mixed cropping conditions. The presence of CMV in the mixed culture reduced the density of NVL, while the opposite was not true. Consequently, the total plant density of the mixed culture was higher than that in CMV. Fox-tail (*Alopecurus aequalis* Sobol) was the most prevalent weed in the winter legume crop (Fig. 1b). Fox-tail density decreased in response to the increase in the legume crop density. Thus the amount of weed in the CMV/NLV mixed culture was the lowest, essentially because the high density of legumes was most effective in controlling the growth of fox-tail at the beginning of spring.

The shoot and root dry weight of CMV was larger in both mono- and mixed croppings than that of NLV (Fig. 2), possibly due to the maintenance of a high plant density in CMV, even as biomass per plant increased with continued growth (Fig. 1). In particular, the shoot and root dry weight of CMV increased rapidly after flowering, with the strongest responses occurring in mono-cropped CMV. In contrast, the shoot dry weight of NLV decreased marginally after flowering in both mono- and mixed culture plots. In the mixed plot, although the presence of CMV reduced the growth of NLV, CMV was not affected by the presence of NLV. This growth response was consistent with the plant density data (Fig. 1), and indicated that total shoot and root growth was greatest in the mixed culture treatment.

In most cases, nodule size and numbers were greater in CMV than NLV, although during early growth just after over-wintering, the number of big nodules in NLV was higher than in CMV (Table 2). This effect was most apparent in the mixed culture.

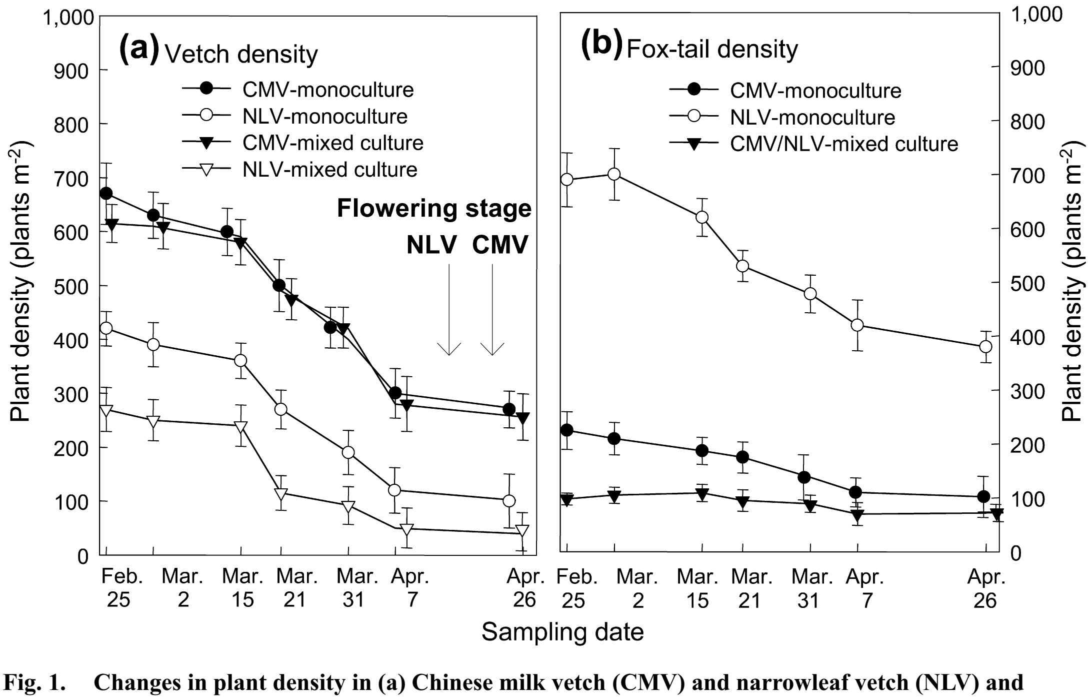

Fig. 1. Changes in plant density in (a) Chinese milk vetch (CMV) and narrowleaf vetch (NLV) and (b) Fox-tail density in CMV and NLV mono- or CMV/NLV mixed culture

Bars indicate the s.e. of the means.

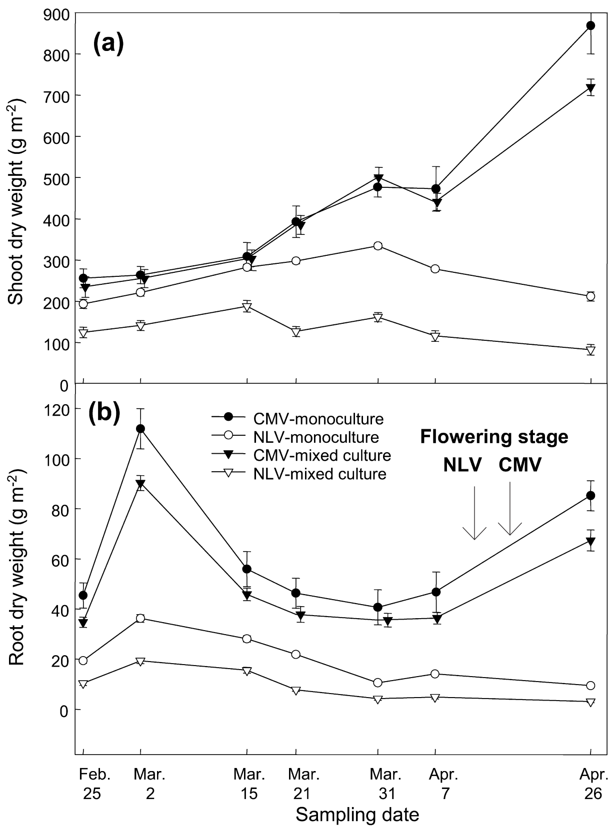

Fig. 2. (a) Shoot and (b) root dry weight in CMV and NLV mono- or mixed culture

Bars indicate the s.e. of the means.

3. Changes in N concentrations (%) and N contents ($g\ m^{-2}$) of vetch shoots and roots

Immediately after over-wintering, shoot and root N concentrations in all the legumes increased, followed by a rapid decline within one week (Fig. 3). Thereafter, shoot and root N concentrations remained relatively stable until flowering. N concentration was not remarkably different between CMV and NLV.

The shoot N content ($g\ m^{-2}$) of CMV in both mono- and mixed cultures increased from 10 to 24 (Fig. 4) in the same way as the response of plant density (Fig. 1) and shoot growth (Fig. 2). The shoot N content of NLV was generally lower than that of CMV (not early in the season in monoculture) and did not increase during the season, while there was a marginal decline in the shoot N content after flowering. The total shoot N content in the CMV/NLV mixed culture was higher than that in either monoculture as the presence of NLV did not generally reduce the shoot N of CMV. The N content in the roots was lower than that of the shoots, ranging from 1.2 to 4.1 $g\ m^{-2}$ in CMV, 0.3 to 1.1 in NLV, and 1.0–3.9 and 0.1–0.5 in CMV/NLV mixed culture, respectively. Although the root N content was more stable than that of shoot N, a marked increase in the root N content of CMV was observed 2 weeks after over-wintering.

4. Effects on nitrogenase activity (NA)

Immediately after over-wintering, the nitrogenase activity (NA) based on the determination of the acetylene

Table 2. Changes in nodule size distribution and numbers per m^2 in CMV and NLV mono- or mixed cultures in a paddy field

Nodule size	Treatment	Date						
		Feb. 25	Mar. 2	Mar. 15	Mar. 21	Mar. 31	Apr. 7	Apr. 26
0–1 mm	CMV-mono	140	472	448	390	344	276	272
	NLV-mono	288	239	188	155	140	126	94
	CMV-mixed	174	264	477	265	168	69	67
	NLV-mixed	314	321	483	465	268	69	13
1–2 mm	CMV-mono	96	144	129	86	66	46	35
	NLV-mono	33	46	50	32	38	9	2
	CMV-mixed	218	436	579	280	193	48	38
	NLV-mixed	96	292	151	140	39	14	2
2–3 mm	CMV-mono	313	152	110	122	147	50	40
	NLV-mono	30	42	46	24	54	5	2
	CMV-mixed	0	18	203	58	58	5	7
	NLV-mixed	61	213	295	0	50	19	2
3 mm >	CMV-mono	0	2	4	5	6	1	1
	NLV-mono	8	20	14	0	7	1	1
	CMV-mixed	140	472	448	390	344	276	272
	NLV-mixed	288	239	188	155	140	126	94

reduction activity (ARA) was 3, 8, 1 and 7 μmol C_2H_4 plant^{-1} h^{-1} in the NLV and CMV mono- and mixed cultures, respectively (Fig. 5a).

However, NA rapidly increased up to 6.4 and 9.1 μmol C_2H_4 plant^{-1} h^{-1} one week later in the NLV mono- and mixed cultures. In mono-cultured NLV, NA reached a maximum value of 10 μmol C_2H_4 plant^{-1} h^{-1} 3 weeks before flowering. NLV flowered one week earlier than CMV. Until flowering, NA in CMV mono- and mixed cultures was 8 and 4 μmol C_2H_4 plant^{-1} h^{-1}, respectively, but it decreased to 3 μmol C_2H_4 plant^{-1} h^{-1} by Mar. 21 in both treatments. Generally, NA was higher in the monocultures than in the mixed cultures throughout the growth period. The content of total fixed N (plant shoot and root N contents minus periodical soil N difference) was 8.6, 4.3, 6.4, and 1.9 g m^{-2} in the CMV-mono, NLV-mono, CMV-mixed, and NLV-mixed cultures, respectively (Fig. 5).

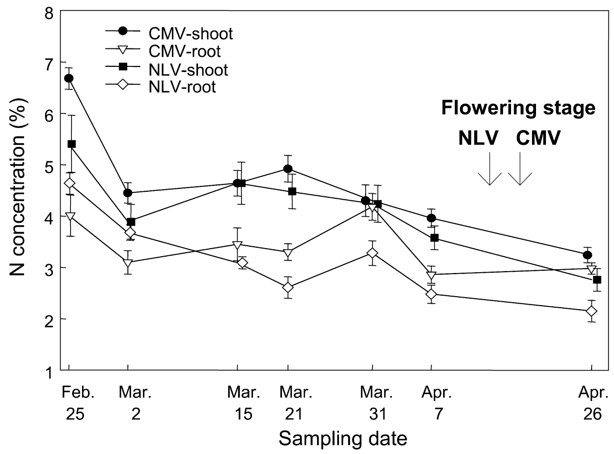

Fig. 3. Shoot and root N concentration (%) in monocultures of CMV and NLV
Bars indicate the s.e. of the mean of 10 measurements.

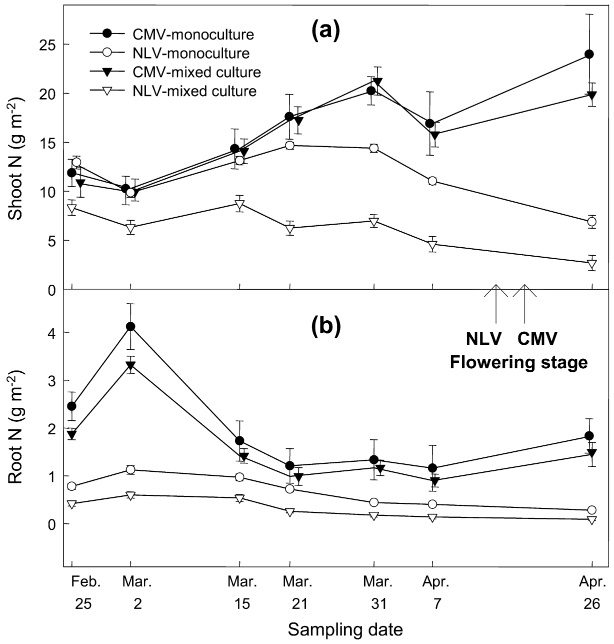

Fig. 4. (a) Shoot and (b) root N contents (g m^{-2}) in CMV and NLV in mono- or mixed cultures of CMV and NLV
Bars indicate the s.e. of the means.

Discussion

A major difference between the 2 vetch species in terms of their value as green manure crops was associated with the density of the plants after over-wintering. Since CMV maintained more plants than NLV throughout the season, the level of shoot and root growth and N content were affected. The lower density of the NLV plants also led to the increase of the growth of fox-tail weeds. Increasing the plant density of NLV in both monoculture and the mixed culture with CMV may alter the results. Even so, the supply of up to 14.5 g N m^{-2} by NLV in this experiment could satisfy the rice requirement for N, since only about 10 g fertilizer N m^{-2} is used in Japanese paddy

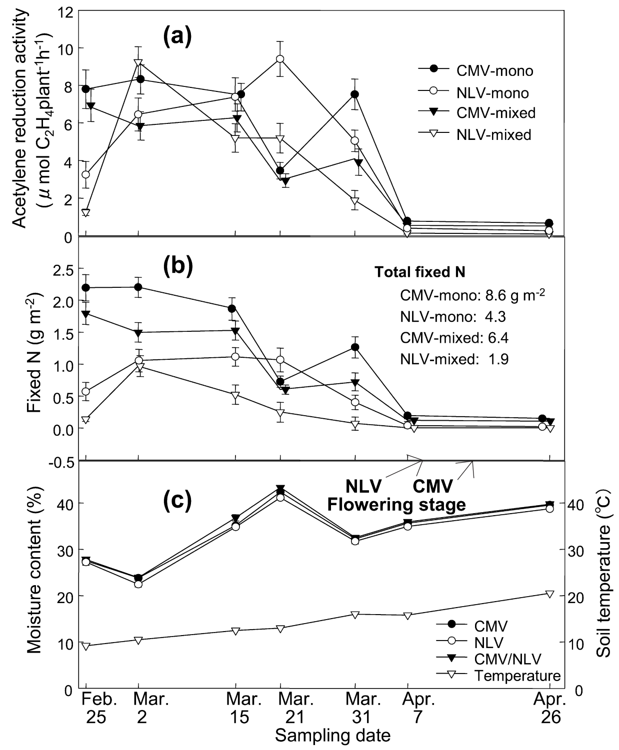

Fig. 5. Changes in (a) nitrogenase activity based on the determination of the acetylene reduction activity (ARA), (b) content of fixed N (g m^{-2}), and (c) soil moisture content (%) and soil temperature (ºC) of CMV and NLV in mono- and mixed cultures
Bars indicate the s.e. of the means.

fields. An important result of this study was that the introduction of NLV into CMV cropping did not reduce the performance of the CMV species. Thus mixed culture resulted in higher plant growth.

Vetch growth responses were generally easy to explain. The shoot N concentration of both CMV and NLV decreased rapidly in the first week after over-wintering. This effect was associated with the rapid growth of the roots and thereafter, there was a constant remobilization of shoot N to root N (Fig. 3). In turn, the increased allocation of growth to roots probably caused both the subsequent increase in nodule numbers (Table 2) and the lag in shoot growth until March 15 (Fig. 2), which was consistent with the fact that the plant density declined only slightly during this period (Fig. 1).

When shoot growth increased in mid-season, particularly in CMV, the root N content became stable. There was a small reduction in the content of soil N from over-wintering to flowering for the CMV and NLV monocultures. A rapid decrease in the soil N content was observed after over-wintering in the CMV/NLV mixed culture, which may be due to the higher combined plant density and shoot growth in the mixed culture. The greater late-season growth of CMV compared with that of NLV may be associated with more adapted growth conditions to the increase of the soil temperature (Fig. 5) or larger amounts of N fixation after the over-wintering period. Due to the larger plant dry weight, CMV produced more green manure N than NLV (Fig. 5b). The value of a green manure is measured both in the amount of atmospheric N fixed, and in the pattern of release of N to a following crop. This eliminates or reduces the need for top-dressing labor. Traditional CMV-rice cropping often induces outbreaks of insect pest populations and diseases in the host plant with a higher N content[7]. Since the N content was mostly correlated with the plant dry weight, the lower N content of NLV may decrease more slowly than that of CMV. Furthermore, the utilization of NLV in rice cropping may also contribute to a decrease in the damage associated with reduction due to the rapid decrease of the oxidation-reduction activity after flooding[4].

The lack of increase of the N contents in shoots and roots of NLV after over-wintering (Fig. 4) indicated that N_2 fixation was very low after wintering in paddy fields, and that most of the plant N uptake occurred either during winter or before winter, although the N uptake during winter is minimal in upland fields[11]. The maximum nodule weight in the NLV mono- or mixed culture was recorded on March 2 or 15. The nitrogenase activity varied with both sampling time and vetch species, suggesting a difference in the response to increased temperature and changes in the soil moisture content (Figs 5a, b and c). The drastic reduction in NA observed in CMV on March 21 coincided with the increase in the soil moisture to 40%, as a result of heavy rain for 3 days before sampling. In this treatment, the moisture did not evaporate because of the high plant density of CMV compared to that of NLV. Inundation may cause a severe reduction of shoot and root growth[17]. The lodging of plants in CMV may have reduced the light penetration and hampered the root activity[8]. Flowering time was also different for CMV and NLV but occurred after both NA and fixation had decreased to minimal levels in both species. In general, the NA values were similar to those reported by Yoshida & Kayama[10]. The amount of fixed N ranged from 2.0 to 8.6 g m^{-2} (Fig. 5b), especially in CMV monoculture, resulting in a higher N-fixing activity (periodically fixed N) than other reported levels[8,12,13], presumably due to the temperature, soil moisture, soil nutrients, and other environmental factors[9,10]. Theoretically, the C_2H_4 to N_2 ratio is 3:1, but in practice, the ratio ranged from 1.5:1 to 25:1[5].

Conclusion

The use of a NLV monocrop instead of CMV might not be recommended, based on biomass production and N_2 fixation. However, the slightly higher C/N ratio in NLV might contribute to sustainable crop production systems. NLV biomass could lead to a maximum N yield of 4.3 g m^{-2}. Even though there was a decrease in NLV growth in the NLV/CMV mixed culture, the total plant and N yield was higher than that in either CMV or NLV monocultures. The system requires further studies to evaluate its beneficial effect on the soil nitrogen supply and residual C/N ratio.

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