Dual and Triple Intercropping: Potential Benefits for Annual Green Manure Production

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Abstract: Greater species diversity in natural ecosystems increases plant biomass production and stability. Intercropping is an agricultural practice that aims to accrue the benefits of species diversity by growing two or more species simultaneously in the same space. Functional group diversity is considered important for enhancing the beneficial effects of species diversity, but most previous intercropping studies used combinations of only two functional groups. Thus, we used three green manure species from different functional groups: sorghum (Sorghum bicolor (L.) Moench.), a C4 grass; crotalaria (Crotalaria juncea L.), a legume; and sunflower (Helianthus annuus L.), a forb. We examined the effects of intercropping on biomass, nutrient uptake, and their stability using a proportional replacement series in a field experiment for three years with four trials. The aboveground biomass was higher with dual and triple-component intercrops compared with sole crops; however, there were no superior effects of triple-component intercropping over dual-component intercropping. There were also no clear advantages of intercropping in terms of the nutrient uptake amount and stability.

Key words: Compensated growth, Nitrogen, Phosphate, Potassium, Replacement design.

It has long been hypothesized that a diverse plant community will be more productive and stable (McNaughton, 1978; Loreau, 2000). Positive relationships between the diversity of plant species and biomass production have been reported in grassland ecosystems (Tilman et al., 1996, 2001, 2006). The mechanisms underlying productivity gains with species diversity are considered to be complementary use of nutrients, water, and light by different species and positive interactions among species (Loreau, 2000). Species diversity has also been correlated with biomass stability (Tilman and Downing, 1994; Tilman et al., 2006). Species differ in their responses to environmental fluctuation; therefore, the species that can resist or adapt to disturbances and new environmental conditions will probably become dominant and their biomass gain will compensate for the biomass loss of susceptible species.

Although previous empirical studies have linked diversity-productivity and diversity-stability relationships in natural plant ecosystems, there is still much debate on the effects of diversity, particularly on diversity-stability relationships (Pfisterer and Schmid, 2002; Van Peer et al., 2004; Ives and Carpenter, 2007). The benefits of species diversity have also been questioned in the field of agriculture where these concepts are applied. In some studies, the forage biomass and its stability were increased over the years by intercropping with an increased numbers of species (Sanderson et al., 2005; Deak et al., 2007; Frankow-Lindberg et al., 2009), whereas other studies revealed no consistent relationship (Sanderson, 2010). These discrepancies among studies are even more pronounced in studies of annual crops because the results are greatly affected by the experimental designs (Connolly, 1988).

Planting density is one of the key factors determining the performance of intercropping compared with sole cropping. The relative ratio of planting density in intercropping to that in sole cropping differs with the...
experimental design, which has produced different results in studies. The most commonly used design is a replacement series, which aims to maintain the same overall densities or proportional densities of intercropping stands as their sole cropping counterparts. There has been much criticism of this design, particularly its inability to analyze inter and intraspecific competition (Connolly, 1988; Connolly et al., 1990; Jolliffe, 2000). However, replacement series may be the most suitable design when addressing a simple question in practical situations (Jolliffe, 2000; Wortman et al., 2012). Without changing total land area and total seed volumes (i.e. without changing the production cost), does mixing species increase biomass and stability?

To increase the beneficial effects of intercropping such as the complementary use of resources and the compensatory effects among component species, we need to increase the functional group (for definition, see Hooper and Vitousek, 1997) diversity rather than the species richness (Loreau, 2000; Kahmen et al., 2006; Sanderson, 2010; Cadotte et al., 2011). However, most intercropping studies based on replacement series have used combinations of only two species. Some recent studies have investigated the biomass and biomass stability of more than two species, but they only included species from 2 functional groups (Wortman et al., 2012; Ma et al., 2013). Other studies have examined the biomass and nitrogen (N) uptake using three species from different functional groups, but either they did not examine the stability (Andersen et al., 2004, 2007) or the seeding rate was much lower than that used in practice (Szumigalski and Van Acker, 2005, 2006).

Thus, we examined the effects of intercropping with three green manure species from different functional groups using a replacement series. The biomass, nutrient uptake, and stability were investigated in a field experiment for 3 years with 4 trials. The species used were sorghum (*Sorghum bicolor* (L.) Moench. cv. Green), crotalaria (*Crotalaria juncea* L. cv. Nemakorori), and sunflower (*Helianthus annuus* L. cv. Russian). Sorghum is a C4 grass species, which is productive at high temperatures and with high irradiance (Loomis and Connor, 1992). Crotalaria, a legume, can reduce interspecific competition for N via symbiotic N fixation under intercropping. Sunflower, a forb, is more productive than C4 species in a low-temperature regime. Forb species also scavenge more N than C4 grass species because they require more N in their photosynthetic apparatuses. Sorghum and crotalaria have relatively deep root distributions compared with sunflower (Miyazawa et al., 2010), which may also facilitate their underground niche differentiation. We hypothesized that if the total seed amount and land area were fixed, intercropping would be advantageous in terms of biomass production, stability, and nutrient uptake compared with sole cropping. We also hypothesized that including a third species from a different functional group would enhance the beneficial effects of intercropping.

**Materials and Methods**

1. **Study site**

A field experiment was conducted at the National Agricultural Research Center for Tohoku Region, Japan (140°23’E, 37°42’N, elevation of 176 m asl). The climate data during the experiments are shown in Fig. 1. The soil was Umbric Andosol (FAO classification), a humus-rich volcanic ash, with a pH of 6.8, a CEC of 31 me 100 g\(^{-1}\), available phosphoric acid of 137 mg P\(_2\)O\(_5\) kg\(^{-1}\), exchangeable potassium (K) of 589 mg K\(_2\)O kg\(^{-1}\), total N of 0.32%, and total carbon of 6.29%. The experimental plots were established in June 2007, where wheat (*Triticum aestivum* L.) had been planted in previous years.

2. **Experimental design**

The experimental design was a randomized complete block with 7 treatments and 3 replicates. The treatments were as follows: three sole crops: sorghum (S), crotalaria (C), sunflower (H); three dual-component intercrops; sorghum and crotalaria (SC), sorghum and sunflower (SH) and crotalaria and sunflower (CH); and one triple-component intercrop (SCH).

The experiment was repeated four times. The seeds of the green manure species were sown on 12 June 2007, 3
August 2007, 10 June 2008, and 12 June 2009; we refer to these trials as J07, A07, J08, and J09, respectively. For J07 and A07, the plot size was 1.5 × 3 m, separated by a 1 m buffer zone. The sites used for these two trials were adjacent. After these two trials, the above biomass was removed, and cattle manure was broadcast to create different soil conditions. The cattle manure, which contained 17 g kg\(^{-1}\) N, 8.3 g kg\(^{-1}\) phosphorus (P), and 18.8 g kg\(^{-1}\) K on a dry weight basis, was incorporated to soil on 10 March 2008 over all of the plots at a rate of 750 g m\(^{-2}\). The experimental plots were rearranged within these sites. For J08 and J09, the plot sizes were 2 × 4.5 m without buffer zone. After the harvest of J08, the green manure crops were incorporated into the soil. Calcium cyanamide (40 g m\(^{-2}\)) was broadcast at the same time over all of the plots to facilitate decomposition and to avoid temporal nitrogen starvation (Hashizume, 2007). In the following fall and spring, cabbage was planted with a fertilization regime of 20 g m\(^{-2}\) N, 17 g m\(^{-2}\) phosphorus pentoside, and 20 g m\(^{-2}\) potassium oxide. After the harvest of spring cabbage, J09 was conducted on the same plot for each treatment.

The seeding rates recommended by the seed companies (sorghum and crotalaria: Snow Brand Seed Co. Ltd; and sunflower: Takii Co. Ltd) were 50 – 60, 60 – 80, and 20 kg ha\(^{-1}\) for S, C, and H, respectively. In J07 and A07, the seeding rates were higher than the recommended rates; 80, 120, and 40 kg ha\(^{-1}\) for S, C, and H, respectively. The density of each species was reduced 1 week after sowing to the rate corresponding to that in J08 and J09, by counting and thinning using 25 × 25 cm\(^{2}\) grids. In J08 and J09, the seeding rates were 60, 80, and 26 kg ha\(^{-1}\) for S, C, and H, respectively. These rates were within those recommended by the seed companies, with the exception of H because a somewhat lower germination rate was expected on the basis of the results of a preliminary germination test. A proportional replacement design was used for the intercropping treatments. The seeding densities of the plants in the dual-component intercrops were half of those in the sole croppings. For the triple-component intercrop, the densities of S and C were each one-fourth of those used in the sole crops whereas that of H was half. All of the seeds were broadcast, mixed with soil using a rotary harrow, and rolled lightly to ensure good contact with the soil.

3. Sampling procedure

The green manure crops were sampled on 23 August 2007, 11 October 2007, 12 August 2008, and 13 August 2009 for J07, A07, J08, and J09, respectively. All of the plants within a randomly placed 50 × 25 cm\(^{2}\) quadrat were cut at the soil surface in J07 and A07, whereas those in a 50 × 50 cm\(^{2}\) quadrat per plot were cut in J08 and J09. The samples were separated according to species, and the fresh weights were measured. Subsamples were dried at 70°C for 48 hr, and the dry weights were measured. The total N content of subsamples were measured using an NC analyzer (Vario Max CN; Elementar Analysensysteme GmbH, Hanau, Germany) after grinding. The total P and K content were determined in J08 and J09 using an inductively coupled plasma spectrometer (VISTA-MPX; Varian Inc., California) after digestion with HNO3 and HClO4 (JSSSPN, 1990).

Soil samples were collected with a boring sampler (3.5 cm in diameter) from depths of 0 – 15 cm at 3 randomly chosen spots per plot just before seeding in each trial, and were sieved through a 2mm mesh. Inorganic nitrogen was extracted from the air-dried soil samples with 2 M KCl and measured with an auto-analyzer (AAII, BL TEC K.K., Tokyo).

4. Data analysis

All statistical analyses were conducted using SAS 9.3 (SAS Institute Inc.). The treatment effect was tested by one-way analysis of variance (ANOVA) using the MIXED procedure with blocks as a random factor; the differences were tested for all combinations of treatments using the adjusted Tukey’s method. To compare sole cropping with intercropping, the treatments were categorized (i.e., nested) as either sole cropping (S, C, and H) or intercropping (SC, CH, SH, and SCH) and were tested using one-way ANOVA with the MIXED procedure and the Type 3 method.

The relative land output (RLO; Jolliffe, 1997) can indicate the relative performance of intercropping compared with sole cropping with the same resource bases (i.e., seed amount and land area). The RLO was calculated as follows:

\[
RLO = \frac{\Sigma Y_j}{\Sigma Y_{ps}},
\]

where \(Y_j\) represents the biomass of “species j” by intercropping, and \(Y_{ps}\) is the proportionate sole cropping biomass of “species j” (e.g., in the case of crotalaria, \(Y_{ps} = Y_i / 2\) in dual-component intercropping, and \(Y_{ps} = Y_i / 4\) in triple-component intercropping; \(Y_i\) represents the biomass of sole cropped crotalaria). A value > 1.0 indicates advantages in terms of the productivity and/or environmental resource use with intercropping. The partial relative land output value (pRLO) was calculated for each species as follows:

\[
pRLO = \frac{Y_i}{Y_{ps}}.
\]

\(Y_i\) is the biomass by intercropping and \(Y_{ps}\) is the proportionate biomass of sole cropping for each species. A value > 1.0 indicates a greater biomass for each species by intercropping than by sole cropping on the basis of an equal amount of sown seed.

Results

The biomass was greater under intercropping than
under sole cropping in J07, A07, and J09 (Table 1). However, compared among all treatments, the aboveground biomass was different only in J09 (Fig. 2). The biomass in C was less than that in S, SH, and SCH. The biomass in CH was also less than that in SH.

Overall, there were no significant differences in the nutrient uptake between sole and intercropping in any trial (Table 1). Among the treatments, the N uptake in S was less than that in CH in J07 and A07 (Fig. 3, $p = 0.0110$ and 0.0216, respectively). The P and K uptakes were different in J09 (Fig. 4). The P uptake in C was less than that in SH and SCH ($p = 0.0124$ and 0.0379, respectively).

### Table 1. Probabilities ($p$-values) from the nested ANOVA based on the differences between cropping methods (sole cropping vs intercropping).

|                | J07   | A07   | J08   | J09   | mean |
|----------------|-------|-------|-------|-------|------|
| Aboveground biomass | 0.0243 | 0.0235 | n.s.  | 0.0246 | 0.0004 |
| N uptake        | n.s.  | n.s.  | n.s.  | n.s.  | n.s.  |
| P uptake        | –     | –     | n.s.  | n.s.  | –    |
| K uptake        | –     | –     | n.s.  | n.s.  | –    |

Fig. 2. Aboveground dry biomass with each treatment in 4 trials. S = sorghum, C = crotalaria, H = sunflower. Different alphabet letters indicate significant differences among the treatments at 5% level by the adjusted Tukey's multiple-range test. Error bars represent the standard deviation of the mean for each treatment.

Fig. 3. Nitrogen uptake in each treatment in 4 trials. S = sorghum, C = crotalaria, H = sunflower. Different alphabet letters indicate significant differences among the treatments at 5% level by the adjusted Tukey's multiple-range test. Error bars represent the standard deviation of the mean for each treatment.
RLO was >1.0 for most of the intercrops throughout the four trials (Table 4), indicating superior effects of intercropping on biomass production over sole cropping using the same amount of seed and land area. pRLO was >1.0 for sorghum in all of the intercrop treatments, whereas other species rarely exceeded 1.0 when intercropped with and the K uptake in C was less than that in H, SH, and SCH (p = 0.0027, 0.002, and 0.0028, respectively). There were no differences in nutrient content within species when compared across treatments. Thus only the average nutrient content in each species across treatments are shown in Table 2 (nutrient uptake was calculated based on the nutrient content values under each treatment). Of the three green manure crops, crotalaria and sunflower had relatively high N contents, and sunflower had the highest P and K content.

The average aboveground biomass across four trials was the highest in SH, followed by SCH and SC (Table 3). The biomass in SH and SCH were higher than that in C (p = 0.0055 and 0.0020, respectively). The average biomass across four trials was higher in intercropping than in sole cropping (Table 1). The biomass stability was the highest (i.e., the coefficient of variation (CV) was the lowest) in H, followed by SH, SCH, and SC (Table 3). The average N uptake across four trials was highest in CH, followed by SCH and H (Table 3). There was no significant difference in N uptake between sole cropping and intercropping (Table 1). The CV value of the N uptake was generally higher (i.e., less stable) than that of the biomass, and it was the highest in CH (Table 3).

Table 2. Nutrient content in the different species across all treatments in 4 trials. S = sorghum, C = crotalaria, H = sunflower. Values are the means ± standard deviations.

|       | N content (g kg⁻¹) | P content (g kg⁻¹) | K content (g kg⁻¹) |
|-------|-------------------|--------------------|-------------------|
|       | J07 | A07 | J08 | J09 | J08 | J09 | J08 | J09 |
| S     | 8.85 ± 1.59 | 17.21 ± 3.30 | 10.52 ± 1.40 | 13.49 ± 2.81 | 2.29 ± 0.46 | 1.89 ± 0.28 | 32.06 ± 3.33 | 30.23 ± 1.99 |
| C     | 21.85 ± 2.52 | 32.44 ± 2.67 | 20.80 ± 2.14 | 25.00 ± 4.25 | 1.80 ± 0.24 | 2.04 ± 0.29 | 24.11 ± 2.37 | 24.15 ± 1.93 |
| H     | 19.24 ± 4.81 | 31.70 ± 3.02 | 17.24 ± 2.99 | 20.72 ± 2.77 | 2.80 ± 0.48 | 2.63 ± 0.15 | 50.51 ± 3.69 | 52.19 ± 3.25 |

Table 3. Mean aboveground biomass and nitrogen uptake in 4 trials and coefficients of variation. S = sorghum, C = crotalaria, H = sunflower. Values are the means ± standard deviations. Different alphabet letters indicate significant differences among the treatments at 5% level by the adjusted Tukey’s multiple-range test.

|       | Aboveground biomass | N uptake |
|-------|---------------------|----------|
|       | mean (g m⁻²) CV(%) | mean (g m⁻²) CV(%) |
| S     | 632ᵃ ± 162 25.6     | 7.5 ± 3.7      49.1 |
| C     | 466ᵇ ± 238 51.1     | 12.5 ± 8.5      67.5 |
| H     | 642ᵃ ± 63 9.8       | 14.3 ± 3.9      27.4 |
| SC    | 717ᵇ ± 123 17.1     | 12.0 ± 5.8      48.7 |
| CH    | 675ᵃ ± 269 39.8     | 17.1 ± 11.7     68.3 |
| SH    | 873ᶜ ± 111 12.7     | 12.9 ± 3.9      30.6 |
| SCH   | 832ᵃ ± 121 14.5     | 14.6 ± 5.9      40.4 |

and the K uptake in C was less than that in H, SH, and SCH (p = 0.0027, 0.002, and 0.0028, respectively). There were no differences in nutrient content within species when compared across treatments. Thus only the average nutrient content in each species across treatments are shown in Table 2 (nutrient uptake was calculated based on the nutrient content values under each treatment). Of the three green manure crops, crotalaria and sunflower had relatively high N contents, and sunflower had the highest P and K content.

Fig. 4. Phosphorus and potassium uptakes in each treatment in 2 trials. S = sorghum, C = crotalaria, H = sunflower. Different alphabet letters indicate significant differences among the treatments at 5% level by the adjusted Tukey’s multiple-range test. Error bars represent the standard deviation of the mean for each treatment.

Table 2. Nutrient content in the different species across all treatments in 4 trials. S = sorghum, C = crotalaria, H = sunflower. Values are the means ± standard deviations.

|       | N content (g kg⁻¹) | P content (g kg⁻¹) | K content (g kg⁻¹) |
|-------|-------------------|--------------------|-------------------|
|       | J07 | A07 | J08 | J09 | J08 | J09 | J08 | J09 |
| S     | 8.85 ± 1.59 | 17.21 ± 3.30 | 10.52 ± 1.40 | 13.49 ± 2.81 | 2.29 ± 0.46 | 1.89 ± 0.28 | 32.06 ± 3.33 | 30.23 ± 1.99 |
| C     | 21.85 ± 2.52 | 32.44 ± 2.67 | 20.80 ± 2.14 | 25.00 ± 4.25 | 1.80 ± 0.24 | 2.04 ± 0.29 | 24.11 ± 2.37 | 24.15 ± 1.93 |
| H     | 19.24 ± 4.81 | 31.70 ± 3.02 | 17.24 ± 2.99 | 20.72 ± 2.77 | 2.80 ± 0.48 | 2.63 ± 0.15 | 50.51 ± 3.69 | 52.19 ± 3.25 |

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| SH    | 873ᶜ ± 111 12.7     | 12.9 ± 3.9      30.6 |
| SCH   | 832ᵃ ± 121 14.5     | 14.6 ± 5.9      40.4 |
There was no significant difference in the concentration of soil inorganic N among treatments, which was 0.82 ± 0.09, 1.70 ± 0.15, 3.21 ± 0.74, and 19.9 ± 8.14 mg N kg⁻¹ in J07, A07, J08, and J09, respectively (average across all the plots ± standard deviation).

**Discussion**

Intercropping green manure species was advantageous in terms of the biomass production across four trials. The intercropping treatments that included sorghum (SH, SCH, and SC) were particularly productive, and biomasses were also stable. Sorghum grew vigorously in all intercrop treatments, as demonstrated by its biomass and pRLO (Fig. 2, Table 5). In a previous study, we found that sorghum developed roots faster than the other two species, and it also developed deeper roots when intercropped than when sown alone (Miyazawa et al., 2010), which may have made sorghum the best contributor to intercropping productivity.

The CH treatment, which did not include sorghum, had the lowest average biomass across four trials among all the intercropping treatments because of the poor growth of crotalaria particularly in J09 (Fig. 2). Crotalaria growth may have been depressed because of the replant disorder. Unlike J07 and A07, all treatments were repeated at the same plots in J08 and J09. The growth of crotalaria became slower than usual, and the leaves of several plants became yellowish in J09. The increase in soil inorganic N concentration may have further depressed crotalaria growth. After the J08 trial, calcium cyanamide was incorporated in all plots, and cabbage production was conducted. These practices increased the inorganic N content of soil in the experimental plots. In previous studies, the growth of intercropped peas was suppressed when N fertilizer was applied, because legumes are less competitive in soil with a high nutrient content (Andersen et al., 2004; Ghaley et al., 2005; Neumann et al., 2007).

Of all the treatments, SH had the highest biomass production and stability; the SCH triple-component intercropping was the second best. Therefore, adding a third species did not increase the productivity and/or stability in our experiment. The proportional density assigned to sorghum and crotalaria in SCH was only one-fourth each, whereas that to sunflower was half of that in sole cropping. Sorghum was the main contributor to the biomass increase in SCH, and it had the highest pRLO in SCH in all trials; however, the low proportional density assigned to sorghum in SCH made it difficult for it to exceed the dual-component intercrops. Andersen et al. (2004) examined the effects of intercropping using 3 annuals, i.e., spring barley (Hordeum vulgare L.), field pea (Pisum sativum L.), and oilseed rape (Brassica napus L.), and also found no superior effects of triple-component intercrops over dual-component intercrops. Moreover, none of the intercrops were more productive than the most productive sole crop. They applied row seeding of mixed seeds, which tended to lower the biomass compared with broadcast sowing (Iqbal et al., 2012), probably because the short interplant distance caused intense competition (Vandermeer, 1992; Iqbal et al., 2012).

**Table 4.** Relative land output (RLO) values for intercrops. S = sorghum, C = crotalaria, H = sunflower.

|       | J07 | A07 | J08 | J09 |
|-------|-----|-----|-----|-----|
| SC    | 1.40| 1.19| 1.20| 1.57|
| CH    | 1.20| 1.50| 1.08| 0.97|
| SH    | 1.42| 1.43| 1.25| 1.39|
| SCH   | 1.31| 1.47| 1.25| 1.60|

**Table 5.** Partial relative land output values (pRLO) for each species in the intercrops. S = sorghum, C = crotalaria, H = sunflower.

|       | J07 | A07 | J08 | J09 |
|-------|-----|-----|-----|-----|
| SC    |     |     |     |     |
| S     | 2.30| 1.59| 1.56| 1.88|
| C     | 0.76| 0.73| 0.69| 0.04|

| CH    |     |     |     |     |
| C     | 1.31| 2.26| 0.56| 0.44|
| H     | 1.10| 0.68| 1.42| 1.09|

| SH    |     |     |     |     |
| S     | 2.78| 2.33| 1.55| 1.81|
| H     | 0.56| 0.27| 0.94| 0.95|

| SCH   |     |     |     |     |
| S     | 2.94| 3.23| 2.73| 2.83|
| C     | 0.42| 1.63| 0.25| 0.18|
| H     | 1.19| 0.25| 0.90| 1.07|
of component species might alter the performance of intercrops. More experiments including different species with different seeding density and proportions are necessary to further generalize this trend.

Based on our results, dual-component intercropping with sorghum and sunflower was the best choice in terms of biomass production and stability (Table 3). Sorghum and sunflower grew well across different climate and soil inorganic N conditions. Relatively low precipitation during the latter half of the experimental period in A07 might be the cause of low pRLO of sunflower in A07 (Table 5); sunflower has relatively shallow rooting pattern and may have been imposed greater water deficiency under intercropping (Miyazawa et al., 2010). The depression of sunflower biomass was well compensated by the increase of sorghum biomass. However, the superior effects of SH may only occur in conditions where sorghum grow well. If sorghum failed to grow, which happened in our previous experiment for unidentified reasons (Miyazawa et al., 2011), it may be difficult for sunflower alone to compensate for the biomass deficit. Although the biomass of sunflower was stable throughout all four trials, biomass growth compensation by sunflower was rarely observed, unlike other two species whose pRLO values reached more than 2.0 when the other species had poor growth (Table 5). Because of the shallow root distribution, sunflower cannot benefit from the resources in deeper soil layers if other component species with deep root systems fail to grow. Thus, sunflower may have been unable to compensate for the biomass loss of other component species.

The beneficial effects of intercropping on nutrient uptake were less obvious compared with those on biomass production. The CH treatment had the highest N uptake in J07 and A08; however, its stability was the least (Table 3). The actual uptake of soil N by CH was also much lower than the observed N uptake, because most of the N recovered from crotalaria is considered to be acquired via symbiosis (Andersen et al., 2004). The N uptake by all other intercrops was also not higher than those by the sole crops. Although intercrops had a high biomass, much of their N gains were canceled out because of the low N content of sorghum. The P and K uptakes were relatively high by SH, SCH, and H, because sunflower had relatively high P and K content (Table 2). The nutrient uptake by intercropping depended on the composition and the dominance of the component species, and a greater biomass was not necessarily coupled with a higher nutrient uptake from the soil. No superiority in nutrient uptake by triple-component intercropping was observed. These results agree with those reported in 2 other triple-component studies using annuals from different functional groups (Andersen et al., 2004; Szumigalski and Van Acker, 2006).

Our hypothesis was partly supported; intercropping was advantageous in terms of biomass production and biomass stability compared with sole cropping, when the total seed amounts and land area were fixed. However, there were no clear advantages in the amount and stability of nutrient uptake. There was also no evidence of extra benefit by intercropping with a third species. If the intercropping performance is improved by a single specific species (i.e. sorghum in our experiment), adding more species may lower the density of the specific species and diminish the benefits of intercropping. Nonetheless, triple-component intercropping was the second best in terms of biomass production and stability, and it was not statistically different from the most productive dual-intercrop. Environmental factors such as climate and soil fertility can affect the performance of particular species drastically during intercropping (Lambers et al., 2004), and unexpected poor growth of specific species can occur such as the cases of crotalaria in this study and sorghum in our previous study (Miyazawa et al., 2011). The fluctuations of environmental conditions, which are anticipated within the context of climate change, could also alter species growth patterns and interactions among species. If prior information is not known about the growth performance of species at a site and/or further environmental variation is expected, adding a third species with a different response to the environment may help to reduce the risk of biomass reduction.

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