Artificial Diversity of Plant–Insect Communities and Modern Crop Stoichiometry in Small Closed Patches in Greenhouse

M. Soufbaf[1], Y. Fathipour[1*], and C. Hui[2]

ABSTRACT

Little is known about the effect of artificial diversity of plant – insect communities on the carbon and nitrogen stoichiometry, weight, and water content of the modern crops. Using a microcosm experiment with two closely related crop species (*Brassica napus* and *B. juncea*), the sap feeder turnip aphid (*Lipaphis erysimi*), the folivore diamondback moth (*Plutella xylostella*) and its larval-pupal parasitoid wasp, *Diadegma semiclausum*, the Shannon biodiversity index was evaluated and regressed to the experimental data of carbon and nitrogen stoichiometry, water content and weight of the crops. Carbon: Nitrogen (C:N) ratio of the modern cultivar under single planting had a logarithmic relationship with the artificial biodiversity index, while this relationship under plant interference was linear and positive. Water content of both experimental crops changed with the artificial biodiversity index conversely under single planting setup. When insects (either the folivore or the phloem feeder) damaged the host plants, the weight and water content of both crop species were 1.8 – 4.1 times higher than the control treatment. Apart from being a recurrent demonstration of the plant tolerance against insect feeding activity, current results can take a step forward for developing a theory on functional artificial biodiversity after herbivore insect–crop interactions.

Keywords: C:N balance, Diamondback moth, Species richness, Shannon index, Stoichiometric analysis.

INTRODUCTION

Biodiversity and ecosystem functioning causal relationship is one of the core subjects in ecology (Loreau, 2010). Against natural biodiversity in which all living organisms interact within an ecosystem, simplified agroecosystems' biodiversity (viz. artificial biodiversity) raises from limited number of plants and animals that are further reinforced by using chemical pesticides. Concentrations of Carbon (C) and Nitrogen (N) in plants have often been reported to show the nutrient level in both managed and natural ecosystems (Fagan and Denno, 2004). Recent claims on the global elemental imbalance in the modern plants and its effect on the elemental composition of plants have been proven relatively well (Loladze, 2002). However, apart from an overall decline in nitrogen concentration, little is known about the effects of artificial biodiversity of agro–ecosystems on carbon and nitrogen stoichiometry, biomass, and water content of the modern plants.

Agriculture, itself, is the biggest manipulation in nature leading to simplified agroecosystems in which biodiversity is artificial. Diversity indices can be used to describe communities' numerical structure

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[1] Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Islamic Republic of Iran.
[*] Corresponding author; email: fathi@modares.ac.ir
[2] Center for Invasion Biology, Department of Mathematical Sciences, Stellenbosch University, Matieland 7602, and Mathematical and Physical Biosciences, African Institute for Mathematical Sciences, Cape Town 7945, South Africa.

better than the number of species present; and we know that a big fraction of communities in the universe are fragmented by human and are then *diversified artificially*. Nevertheless, species flow among local communities and a regional species pool is important confidently and is also tested by many researchers (Wilsey and Stirling, 2007; Matthiessen *et al.*, 2007). Researchers suggest that species richness can affect species function in closed systems through net biodiversity effects. Here, we are trying to show the biodiversity effects on insect related plant attributes in closed systems. Moreover, one of ecological issues behind diversity indices is about relationships between richness and evenness and, certainly, interactions in insect-plant communities are steady.

Here, we aimed to apply stoichiometric theory to argue that rising species richness and its subsequent artificial biodiversity may alter the elemental composition of the modern plants favorably. Moreover, we hypothesized that plant interspecific interference affects the modern plant stoichiometry–artificial biodiversity co–dynamics again favorably as the increased plant density is one of modules of the raised artificial biodiversity. Then, we examined the plants' characteristics to show that whether these attributes have any trend toward artificial biodiversity dynamics. Importantly, we only considered this kind of interference due to its role in polycultures; while through monoculture, plants forcibly experience intraspecific interference. Further, we argued that species richness should not be studied singly because the function of the experimental species is also determinant. Therefore, we examined two important insect feeding modes including folivory vs. sap feeding that might alter the co–dynamics in question differently. Specifically, the diamondback moth, as a specialist folivore insect and specialist phloem feeder insect, the turnip aphid, were used to induce different crop responses because of their versatile feeding tactics.

Moreover, to increase the system richness we considered the effect of introducing pest parasitism, an important part of a typical agricultural tritrophic system that can indirectly affect crop performance.

MATERIALS AND METHODS

A modern crucifer cultivar, *Brassica napus* L. cv. SLM$_{046}$, (R1) and a landrace cultivar, *Brassica juncea* L. (R2) were used in the experiment. The seeds of both crucifer cultivars were obtained from the Seed and Plant Improvement Institute (Karaj, Iran). Colonies of *Plutella xylostella* L. (h1), and *Lipaphis erysimi* (Kaltenbach) (h2), were established and kept in the laboratory on cabbage, *B. oleracea* var. *capitata* L.. The stock cultures were maintained for 60 days in the greenhouse before the experiment (Soufbaf *et al.*, 2010a, 2010b, 2012). The parasitoid, *Diadegma semiclausum* (Hellén) (p) was reared on *P. xylostella* larva in a constant environment at $25\pm2°C$, and adults of the second generation were used in the experiment.

Experimental plants were grown in 15×20 cm pots under greenhouse condition ($27\pm5°C$, $60\pm10\%$ RH) and the number of individual plants was the same in all treatments (one plant for each species). The soil was sterilized using 30 kGray gamma radiation to ensure the removal of all soil microorganisms. Plants were irrigated daily and caged in a good ventilation transparent plastic cage i.e. a closed patch ($40\times40\times50$ cm). The dead leaves of the experimental plants were removed daily from the cage and insect feces were discarded weekly. Two female folivore insects (who were left for mating with two males for 24 hours in a small cage), three 4th instar nymphs of the phloem feeder (picked by a fine brush), and two mated female parasitoids (2 female and 2 male wasp were vacuumed with an oral aspirator from the stock colony of the parasitoid and were left for 24 hours in a glass vial to mate), were released into

respective cages to establish different cropping systems.

Combined effects of the two different crop species (R1, R2) and plant interspecific interference (R1+R2) under insect herbivory (h1, h2), herbivore coexistence (h1+h2), insect parasitism (h1+p) and herbivore coexistence plus parasitism (h1+h2+p) on the stoichiometry, weight and water contents of the experimental crops were studied in the experimental transparent cages (above). The Shannon diversity index (H´), was used to quantify the artificial biodiversity in each treatment:

$$H' = -\sum_{i=1}^{R} P_i \times Ln\, P_i$$

Where, P_i is the Proportion of individuals belonging to the ith species in each treatment and R is total Richness of all species in each treatment, i.e. total number of all plant and insect species in each cage. Abundance of each insect species in each treatment was averaged over the replication number and then was divided by total abundance of all insect and plant species to get the proportion of individuals of each species. Proportion of each species and its Naperian logarithm was multiplied for each treatment and their summation with a negative sign was considered as the Shannon index. Explanation of the 18 different model systems is represented in Table 1. We managed all cages for an entire season of the crops and all data were recorded at the end of the season. The nitrogen and carbon contents of crop root and foliage were examined by a CHN analyzer (vario EL III, CHNOS elementar, Germany) after oven drying of each cultivar at 55°C for 12 hours. Dry weight was measured using a Sartorius electronic balance (GMBH, Göttingen, Germany).

Except for the systems without insects and systems with the phloem feeder insect that were replicated three times, others were replicated six times. We had two levels of plant species (modern *vs.* landrace), two levels

Table 1. Model systems, components, richness, and the Shannon index (H′) of the artificial diversity under greenhouse conditions.

Model systems	System components					SR[f]	CR[g]	H′
	R1[a]	R2[b]	h1[c]	h2[d]	P[e]			
1	+					1	1	0.346
2	+		+			2	1	0.094
3	+			+		2	1	0.045
4	+		+		+	3	1	0.633
5	+		+	+		3	1	0.255
6	+		+	+	+	4	1	0.180
7		+				1	1	0.346
8		+	+			2	1	0.043
9		+		+		2	1	0.011
10		+	+		+	3	1	0.279
11		+	+	+		3	1	0.355
12		+	+	+	+	4	1	0.478
13	+	+				2	2	0.693
14	+	+	+			3	2	0.100
15	+	+		+		3	2	0.014
16	+	+	+		+	4	2	0.371
17	+	+	+	+		4	2	0.660
18	+	+	+	+	+	5	2	0.109

[a] Modern crucifer cultivar *Brassica napus*, cv. SLM$_{046}$; [b] Landrace cultivar *B. juncea*; [c] Specialist folivore insect pest, *Plutella xylostella*; [d] Specialist phloem feeder insect pest, *Lipaphis erysimi*; [e] Larval parasitoid of h1. [f] System richness, represents the total number of initial species in each treatment; [g] Crop richness, represents the number of crop species in each model system.

of plant density (mono *vs*. mixed), two levels of plant organs (shoot *vs*. root), and five levels of insect herbivores. For plant elemental chemistry variation among different organs, cropping systems and crop species, effect of insects was ignored and data were reanalyzed. Totally, we had 80 and 75 replicates for modern and landrace plant species, 80 and 75 replicates for mixed and mono planting, and 84 and 71 replicates for root and shoot, respectively. Except for the diversity index values, other data were subjected to the Kolmogorov-Smirnov test for conformity to a normal distribution before analyses. Data on nitrogen, C:N, and shoot water were log–transformed, Lngamma–transformed, and sqrt–transformed to meet normality, respectively, and then were analyzed using multivariate General Linear Model (GLM) after full factorial model with plant species, planting set up, and plant organ as fixed factors. Main effects interactions were significant for the carbon value, therefore, its main effect was reanalyzed through univariate GLM after full factorial model with plant species, planting set up, and plant organ as fixed factors. Means of the variables under the insects effect were compared using LSD test at $P= 0.05$. Regression analyses between artificial biodiversity and crop stoichiometry and weights were performed using the curve estimation technique considering the Shannon index as independent variable, and cases with a missing value in any variable were excluded from the analyses. All analyses were done using SPSS (2008).

RESULTS

The highest Shannon biodiversity index (H´), was calculated in the R1R2 treatment (H´= 0.693) while the lowest value was measured in the R2H2 treatment (H´= 0.011) (Table 1). There were significant differences of carbon content between planting treatments ($F_{1,147}=4.78$, P= 0.03) and between plant organs ($F_{1,147}= 45.49$, P< 0.0001), while crop species had no effect on the carbon content ($F_{1,147}= 3.02$, P= 0.084) (Figure 1-a). However, there were significant differences of nitrogen between crop species ($F_{1,153}=19.54$, P< 0.0001), planting treatments ($F_{1,153}= 19.28$, P< 0.0001), and plant organs ($F_{1,153}= 20.48$, P< 0.0001) (Figure 1-b). C:N ratio was different between crops ($F_{1,153}= 22.08$, P< 0.0001), planting treatments ($F_{1,153}= 20.7$, P< 0.0001), and plant organs ($F_{1,153}= 45.69$, P< 0.0001) (Figure 1-c).

Root dry weight ($F_{5,81}= 2.54$, P= 0.035) (Figure 2), root water content ($F_{5,81}= 4.82$, P< 0.001) (Figure 3) and foliage water content ($F_{5,81}= 2.51$, P= 0.037) (Figure 4) were affected by the insect treatment, while plant treatment only affected the foliage water content ($F_{1,81}= 6.02$, P= 0.016) (Figure 4). Planting treatment affected foliage dry weight ($F_{1,81}= 7.47$, P= 0.008), root dry weight ($F_{1,81}= 7.06$, P= 0.009), and root water content ($F_{1,81}= 10.35$, P= 0.002) (Figures 5, 2, and 3, respectively). No significant difference of the root dry weight ($F_{1,81}= 0.53$, P= 0.47), foliage dry weight ($F_{1,81}= 2.61$, P= 0.11), and root water content ($F_{1,81}= 0.92$, P= 0.34) was observed between two plant treatments. The highest root dry weight was recorded in the mono cultivation treatment, while the lowest root dry weight was recorded in the mixed cultivars (Figure 2). Root water content was the highest when both insect herbivores acted in concert under mono cultivation (Figure 3). The highest foliage water content was recorded in the treatment of modern crucifer cultivar, while the lowest foliage water content was recorded in the landrace cultivar (Figure 4). Single planting (one individual) showed higher values of the crop weight than mixed planting of both cultivars (two mixed individuals). The highest foliage dry weight was observed in the treatment with mono cultivation, while there were no significant differences among different insect treatments (Figure 5).

While the landrace cultivar stoichiometry showed no relationship with the artificial biodiversity, there was a logarithmic
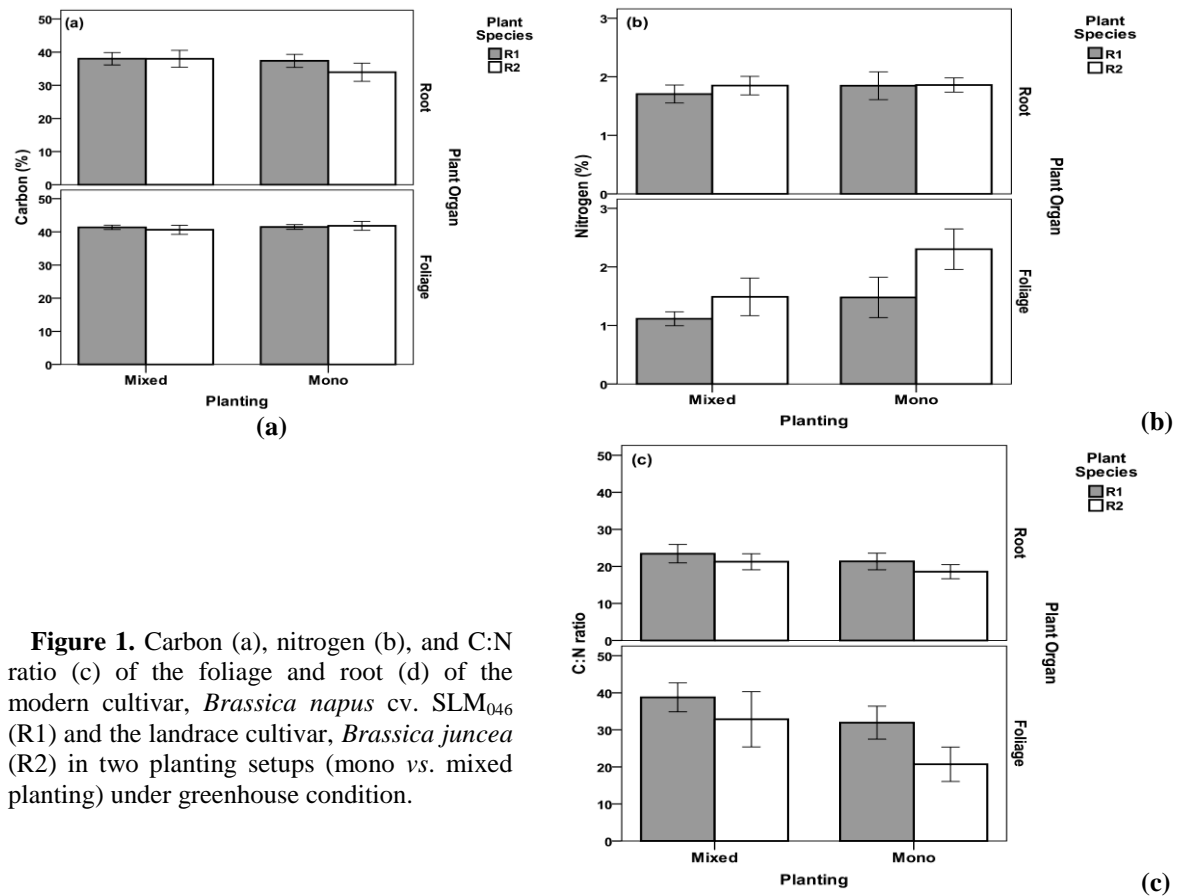
Figure 1. Carbon (a), nitrogen (b), and C:N ratio (c) of the foliage and root (d) of the modern cultivar, *Brassica napus* cv. SLM$_{046}$ (R1) and the landrace cultivar, *Brassica juncea* (R2) in two planting setups (mono *vs.* mixed planting) under greenhouse condition.
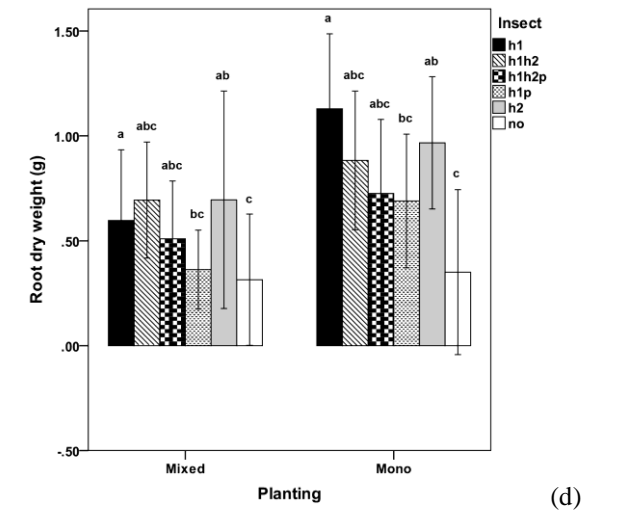
Figure 2. Root dry weight of the experimental crop species, *Brassica napus* cv. SLM$_{046}$, and *Brassica juncea* under effects of five different insect treatments and two different planting setups under greenhouse condition. h1, h2, h1h2, h1h2p, h1p represent the folivore insect, phloem feeder, coexistence of both insect herbivore species, coexistence of h2 with the parasitized h1, and the coexistence of h1 with its parasitoid, respectively. The letters on each error bar represent the insect guild mean comparisons after LSD test at *P*< 0.05, respectively

relationship between the modern cultivar C:N ratio and artificial biodiversity in R1 treatment ($F_{1,4}$= 8.21, P= 0.046) (C:N=-6.92×Ln(H′)+20.34, R^2_{adj}= 0.59) (Figure 6-a, framed plot). However, similar to landrace cultivar, carbon and nitrogen of the modern cultivar showed no correlations with the artificial biodiversity in R1 treatment. C:N ratio of the modern cultivar changed linearly with
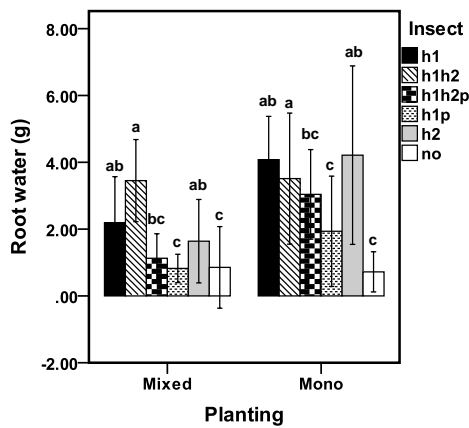
Figure 3. Root water of the experimental crop species, *Brassica napus* cv. SLM$_{046}$, and *Brassica juncea* under effects of five different insect treatments (Insects symbols as defined previously). The letters on each error bar represent the insect guild mean comparisons after LSD test at *P*< 0.05, respectively.
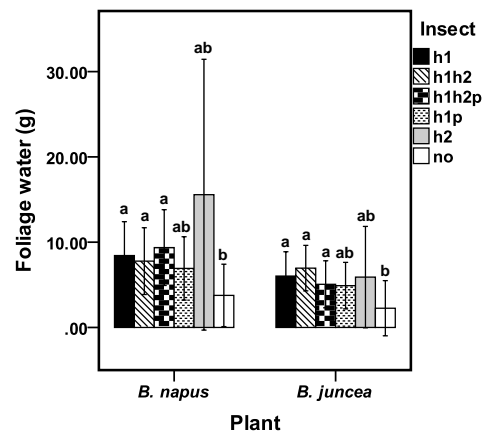

Figure 4. Foliage water content of the two experimental crop species, *Brassica napus* cv. SLM$_{046}$, and *Brassica juncea* under effects of the five different insect treatments (Insects symbols as defined previously). The letters on each error bar represent the insect guild mean comparisons after LSD test at *P*< 0.05, respectively.
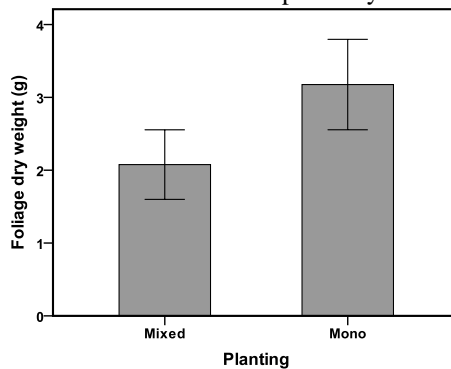

Figure 5. Foliage dry weight of the experimental crop species under two different planting setups (mono vs. mixed planting) under greenhouse condition.

artificial biodiversity index ($F_{1,4}$= 8.16, P= 0.046) (C:N=15.13×H′+33.38, R^2_{adj}= 0.59) (Figure 6-b, framed plot) in R1R2 treatment. However, there were significant relationships between both R1 carbon [$F_{1,4}$= 5.55, P= 0.078, R^2_{adj}= 0.48, Model: carbon= -0.019×(1/H`)+41.61] and nitrogen [$F_{1,4}$= 4.81, P= 0.093, $R^2_{adj}$= 0.43, Exponential model: Ln(nitrogen)= -0.372×H`-1.26] and artificial biodiversity index at 10% significance level in R1R2 treatment. Among weight and water features of the modern cultivar that were measured, foliage water content had a logarithmic relationship

with artificial biodiversity index in R1 treatment ($F_{1,4}$= 7.29, P= 0.054) (water=-8.06×Ln(H′)+1.46, R^2_{adj}= 0.56) (Figure 7-a, framed plot). Similarly, the modern cultivar foliage water showed a S–shape relationship with artificial biodiversity index in R1R2 treatment ($F_{1,4}$= 9.05, P= 0.04) (Ln(water)=-0.014×(1/H′)+2.14, R^2_{adj}= 0.62) (Figure 7-b, framed plot).

There was a *S*–shape relationship between the modern cultivar root dry weight and artificial biodiversity index in R1R2 treatment [$F_{1,4}$=19.93, P= 0.011, R^2_{adj}= 0.79, S–curve model: Ln(root weight)= -
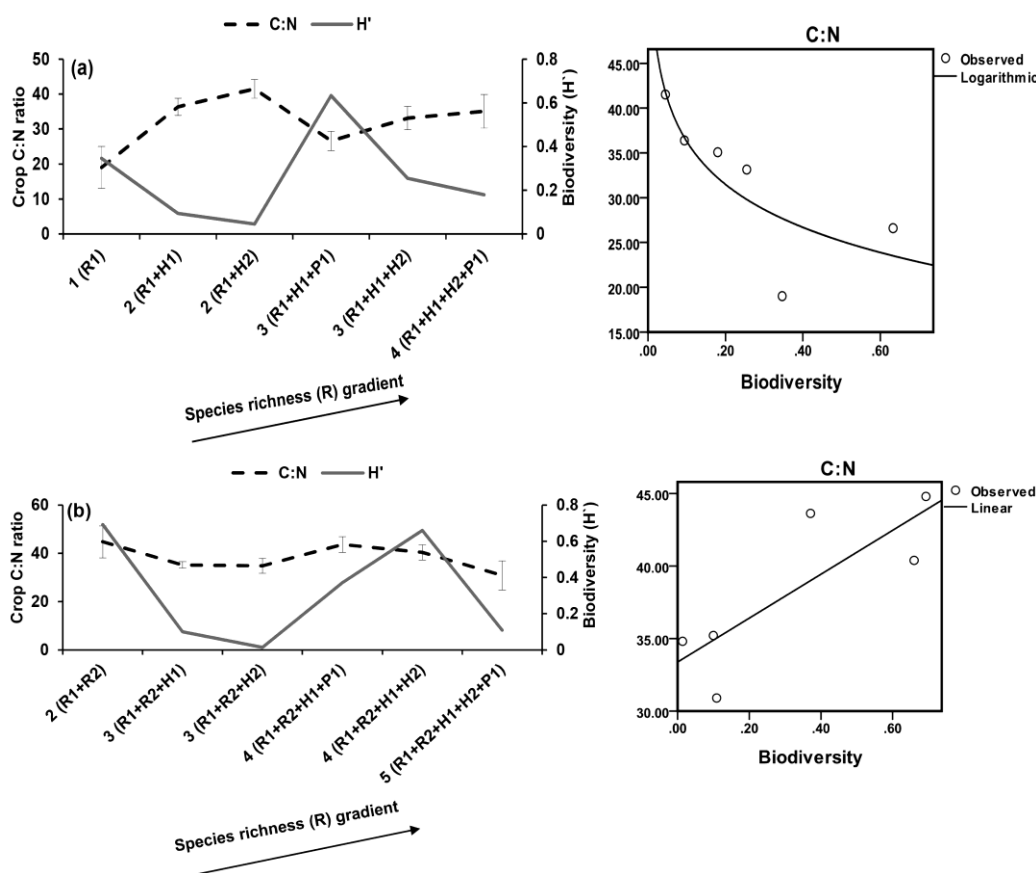
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Figure 6. C:N ratio of the modern cultivar with the Shannon biodiversity index (H′) in R1 (a) and R1R2 treatment (b). H1, H2, and P1 are *Plutella xylostella*, *Lipaphis erysimi*, and *Diadegma semiclausum*. Species richness change with the crop stoichiometry variations was not significant and is portrayed on the horizontal axis just to give a snapshot of variations in question with richness.

$0.007 \times (1/H`) - 0.398$]. However, there was a power–law relationship between the modern cultivar foliage dry weight and artificial biodiversity index at a 10% significance level [$F_{1,4}= 4.95$, P= 0.09, $R^2_{adj}=0.44$, Model: Ln(foliage weigh)= $0.2 \times Ln(H`)+3.36$]. The landrace cultivar foliage water in both R1 and R1R2 treatments was correlated with artificial biodiversity index. In R1 treatment, there was a linear relationship between shoot water and artificial biodiversity ($F_{1,4}= 29.09$, P= 0.006) (water=$-25.38 \times H′+20.06$, $R^2_{adj}= 0.85$) (Figure 8-a, framed plot) while it was inverse in R1R2 treatment ($F_{1,4}= 8.91$, P= 0.041) (water=$0.1 \times (1/H′)+3.9$, $R^2_{adj}= 0.61$) (Figure 8b, framed plot). Root water of the landrace cultivar changed with the artificial

biodiversity linearly at 10% significance level in R2 treatment [$F_{1,4}= 5.87$, P= 0.073, $R^2_{adj}=0.49$, Model: Root water= -$11.49 \times (H`)+6.55$]. In R2 treatment, foliage dry weight of the landrace cultivar changed with the artificial biodiversity index, following a cubic relationship at 10% significance level [$F_{3,2}= 14.55$, P= 0.065, $R^2_{adj}=0.89$, Model: Foliage weight= $61.7 \times (H`)-314.83 \times (H`)^2+391.13 \times (H`)^3+2.56$]. Also, there was a negative relationship between foliage dry weight of the landrace cultivar and artificial biodiversity index in R1R2 treatment at 10% significance level [$F_{1,4}= 5.49$, P= 0.079, $R^2_{adj}=0.47$, Model: Foliage weight= $0.034 \times (1/H`)+1.5$]. Except for one case in
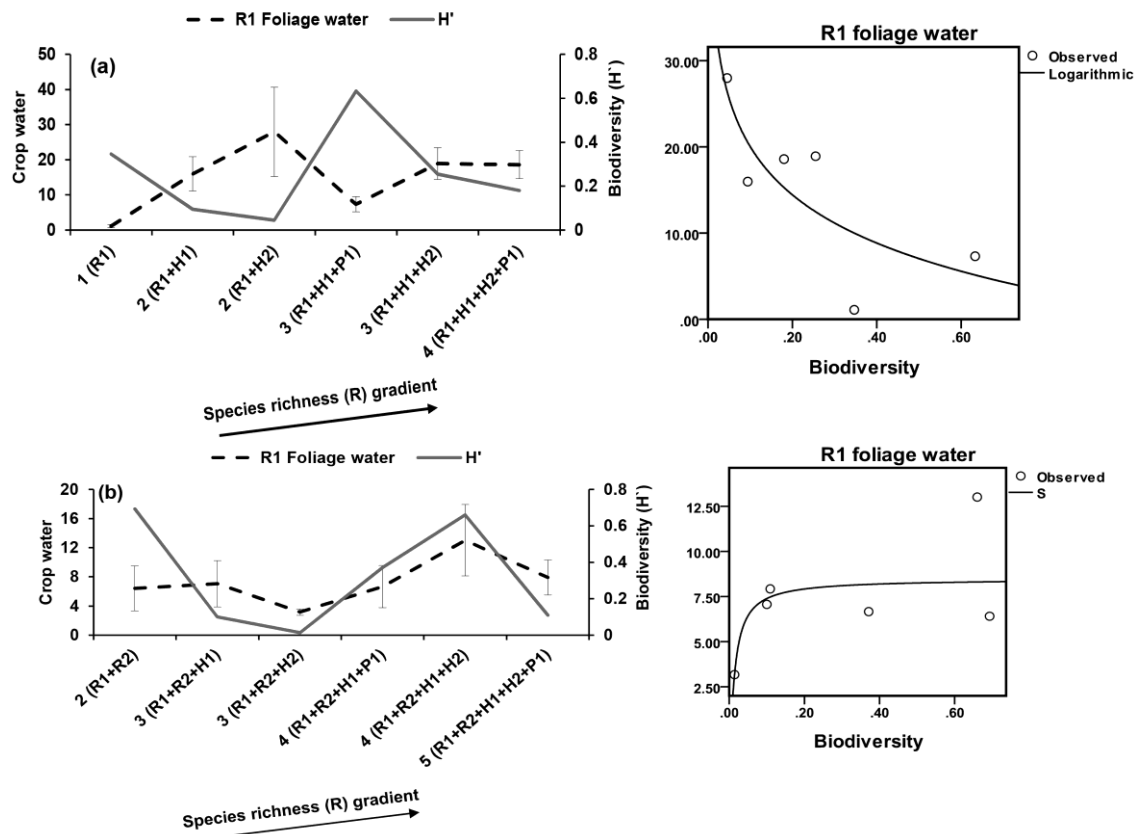
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Figure 7. Water content of the modern cultivar with the Shannon biodiversity index (H′) in R1 (a) and R1R2 treatments (b). H1, H2, and P1 are defined previously. Species richness change with the crop stoichiometry variations was not significant and is portrayed on the horizontal axis just to give a snapshot of variations in question with richness.

which an S shape relationship was found between species Richness (R) and the modern cultivar's foliage water [Figure 7-a; Model: Ln(foliage water)=-3.55(1/Richness)+4.07; $F_{1,4}$= 7.90, P= 0.048, R^2_{adj}=0.58], there was no relationship between the species richness and crops' stoichiometry, water content and biomass.

DISCUSSION

We integrated data from a richness manipulation experiment with curve estimation technique to visualize the governing pattern of the stoichiometry–artificial biodiversity co–dynamics in a modern cultivar of *Brassica napus* and a landrace cultivar of *B. juncea* under different insect assemblages. We found that different insect compositions had the same effect on the crop weight and water content. The results showed that only root weight and water content of both crops were affected by insect herbivory. When the phloem feeder damaged the crops, wet weight, dry weight, and the water content of the crop roots were 2.95, 1.87, and 3.44 times higher than in the control treatment (no insects). Similarly, when the folivore insect damaged the crops, the wet weight, dry weight, and the water content of the cultivars roots were 3.52, 1.81, and 4.1 times higher than in the control systems. However, crop weight and water contents did not vary significantly among different insect treatments. Thus, it seems that the insect species richness did not affect crop biomass production. The greenhouse data
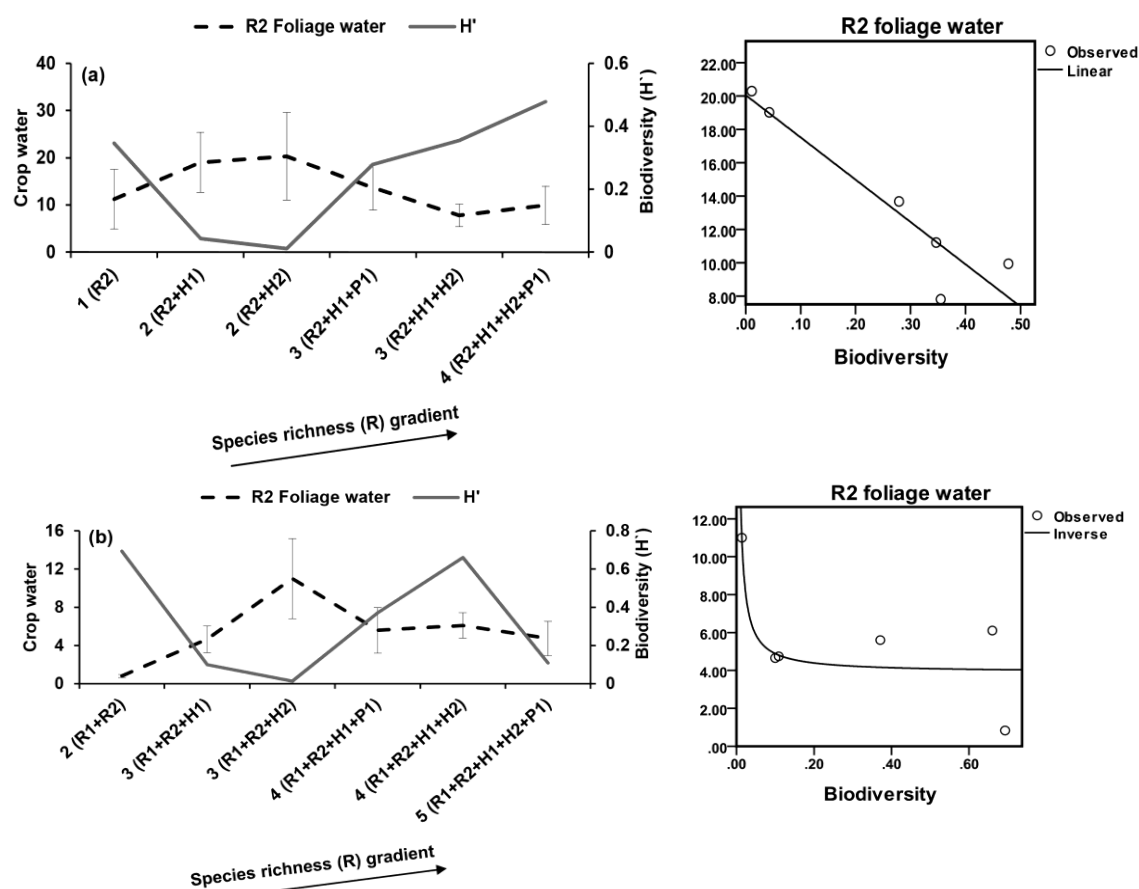
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Figure 8. Water content of the landrace cultivar with the Shannon biodiversity index (H′) in R2 (a) and R1R2 treatment (b). H1, H2, and P1 are defined previously. Species richness change with the crop stoichiometry variations was not significant and is portrayed on the horizontal axis just to give a snapshot of variations in question with richness

showed significant responses of crop stoichiometry to artificial biodiversity and these responses appeared to be influenced by host plant quality as these responses were observed only for the modern cultivar. C:N ratio of the modern crop under single planting had an inverse relationship with the artificial biodiversity index after a logarithmic model, while it was positive and linear under plant interference. As a result, our first hypothesis was supported properly for the biodiversity rather species richness. However, our later claim on the plant interference effect was not proved thoroughly. Considering C:N ratio as an index for plant quality, we observed that this attribute, i.e. plant quality, decreased with biodiversity increase when plant interference

challenged the modern crop. Expectedly, when the modern crop was single, its quality increased after biodiversity increase. There seems to be a contradiction in the role of interfering plant, i.e. landrace cultivar, in increasing biodiversity on one hand vs. its effect on the plant quality decrease on the other hand. Therefore, we concluded that the function of species that shape a biodiversity is more important than just their number (although the quality affects the emanated numbers too). In other words, there could be patches with exactly the same biodiversity (numerically) but with different qualities (functionally).

In the current study, the carbon and nitrogen stoichiometry differed between plant organs.

Without herbivores, the root of modern crucifer cultivar showed the lowest C content while other systems showed non-significant differences in carbon. For N, there was no consistent pattern among different cropping systems, while for the lowest amount of N, the modern crop foliage was the candidate. Further, the highest C:N ratio of the crops were found in the modern crucifer cultivar foliage under mixed planting. Throop *et al.* (2004) showed that N deposition and herbivory had conflicting influences on the N storage because of their contrasting impacts on biomass production. Specifically, N deposition improves N storage in the vegetation by increasing above– and below-ground biomass. Most notably, no regression model was found between the landrace cultivar stoichiometry and artificial biodiversity index, under either single or mixed planting setups.

When the artificial biodiversity index varied, no significant difference was observed in the wet and dry weights of crop cultivars in different treatments; that is, the biomass production did not differ with increasing artificial biodiversity index. Some studies have shown that deliberate management of plant diversity with fertilizers could increase crop biomass e.g. Vance *et al.* (2003). In contrast, other studies have suggested a positive relationship between diversity and biomass in both annual and everlasting plant species e.g. Jiang *et al.* (2007). The results of the present study showed that the dry weights of crop foliage and root were much higher under the single planting set up (low plant richness) than in treatments with mixed planting (high plant richness). However, among different systems tested in the current study, there was a strong and negative relationship between the root weight of the modern cultivar and biodiversity index under mixed planting. The weight and water contents of the single modern crucifer and landrace host plant were higher than in mixed planting. In contrast, Cardinale *et al.* (2007) showed that mixtures of plant species produced an average of 0.7 times more biomass than the monoculture species (at landscape). Cadotte (2013) further showed that

when potted with distantly related species, plants produced more biomass than from their monocultures and the produced biomass predicted from the monoculture when sown with the close relatives. This suggests that combining distantly related species may serve to increase the biomass production and carbon sequestration. One of the most important findings of the current study is that water content of both experimental crops changed with the biodiversity index conversely. Foliage water of the modern cultivar under single planting showed a strong converse regression with the biodiversity index. Similarly, foliage water of the landrace cultivar under both single and mixed plantings showed a strong negative relationship with the biodiversity index. Under single planting, water of both host plants and artificial biodiversity index co–dynamics were inversed. However, under plant interference, the co–dynamics were completely unidirectional. One of probable reasons for this phenomenon is that under plant interference, plants' involvement in the challenge makes them acquire, utilize, and reserve the resources e.g. water, more carefully than when they are single users of available resources. Many studies have suggested that single plant species in a monoculture system have strong influence on ecosystem work as the influence of the single species is more pronounced in ecosystems with low biodiversity index. However, plant diversity had no effect on the productivity of the agroecosystems while it is influenced by the growth stage of individuals (Aguiar *et al.*, 2013; Chapin III *et al.*, 2000).

In conclusion, the current study showed that with increasing artificial biodiversity the crop biomass production did not alter. When insects (either folivores or phloem feeders) attacked the crops, the weight and water contents of both host plants were 1.8–4.1 times higher than in the control treatment. Besides, our data showed that the water content of both experimental crops changed with the artificial biodiversity index conversely under single planting setup. Apart from being a recurrent demonstration on the plant tolerance against insect feeding activity, this finding is a step

forward for developing a theory on emanated artificial biodiversity after herbivore insect–crop interactions. Integrated pest management could also benefit from our results considering different herbivore insect treatments, e.g. sap feeding vs. leaf feeding, acting solely or in concert with competitors or parasitoids, as well as their effects on crop weight and water contents.

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تنوع ساختگی اجتماعات گیاه-حشره و استویکیومتری ارقام گیاهی مدرن در پرگاله های کوچک بسته در گلخانه

م. سوف باف، ی. فتحی پور، و ک. هویی

چکیده

اطلاعات مختصری در زمینه اثر تنوع زیستی اجتماعات گیاه—حشره بر استویکیومتری، وزن و آب ارقام مدرن گیاهی وجود دارد. با استفاده از یک آزمایش میکروکازم با دوگونه گیاهی خویشاوند نزدیک (*Brassica napus* و *B. juncea*)، شته خردل شیره خوار (*Lipaphis erysimi*)، بید کلم برگخوار (*Plutella xylostella*) و پارازیتوئید لارو – شفیره آن (*Diadegma semiclausum*)، شاخص تنوع شانون برآورد شد و به داده‌های آزمایشی مربوط به استویکیومتری کربن و نیتروژن، آب و وزن گیاهان رگرسیون شد. نسبت C:N رقم مدرن تحت کشت تکی یک رابطه لگاریتمی با شاخص تنوع نشان داد در حالی که این رابطه تحت مداخله گیاهی خطی و مثبت بود. محتوای آب هر دو گونه گیاهی در کشت تکی بر عکس تنوع زیستی تغییر کرد. هنگامی که حشرات (چه برگخوار و چه شیره خوار) به گیاهان آسیب زدند، وزن و آب هر دو گونه گیاهی ۱/۸ تا ۴/۱ برابر بیش‌تر از تیمار شاهد بود. جدای از تایید مجدد تحمل گیاهان علیه فعالیت تغذیه‌ای حشرات، نتایج حاضر می‌تواند قدمی برای توسعه یک تئوری در زمینه تنوع زیستی تابعی بر اساس برهمکنش‌های گیاه – حشره گیاهخوار باشد.