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Effect of Differential N and S Competition in Inter- and Sole Cropping of *Brassica* Species and Lettuce on Glucosinolate Concentration

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ABSTRACT: Field and greenhouse pot experiments were conducted to evaluate the potential to use intercropping as an alternative method to increase glucosinolates in *Brassicas* by manipulating nitrogen (N) and sulfur (S) balance by intercropping with lettuce (*Lactuca sativa* L. var. *capitata*). In both experiments, four combinations of N and S fertilization were used. In the field experiment no effect of intercropping on the total glucosinolate concentration was found as the growing lettuce was strongly inhibited by the presence of broccoli (*Brassica oleracea* L. var. *italic*). In contrast to this, in the pot experiment both total and individual glucosinolate concentrations in red leaf mustard (*Brassica juncea* L.) increased by intercropping. Fertilization treatments influenced glucosinolate concentrations in both experiments, and an interaction between N and S fertilization was noticed.

KEYWORDS: *glucosinolates, intercropping, lettuce, mustard, broccoli, nitrogen, sulfur, root growth*

INTRODUCTION

The *Brassica* crops with their high sulfur (S) demand have attracted attention due to the increasing S deficiency in many parts of the world, caused by intensive crop production, reduced atmospheric inputs, and soil characteristics.[1] Sulfur is found in amino acids, oligopeptides, vitamins and cofactors, and a variety of secondary compounds in plants. Glucosinolates (GSLs) are nitrogen (N)- and S-containing plant secondary metabolites found mainly in the order *Brassicales*, and the formation of GSLs is the main reason for the high S demand by *Brassica* crops. The enzymatic degradation products of GSLs contribute to the characteristic flavor of *Brassicas* and their pathogen defense system or serve as insect attractants.[2] In relation to human health, hydrolysis products of certain GSLs are associated with beneficial effects due to their anticancer properties.[2] Glucosinolate concentration and profile are influenced both by genetic and environmental factors.[3−5] In most cases, S supply increases GSL content, which is not surprising, since each GSL molecule contains two or three S atoms. Sulfur fertilization has an impact not only on the total GSL content but also on the accumulation of individual GSLs in different *Brassica* species, for example, *Brassica napus*,[6] *Brassica oleracea* var. *italic*,[7] and *Brassica rapa*.[8]

Studies have shown contradictory effects of N supply and its interaction with S supply, GSL concentration, and composition in plants, and they have indicated that to enhance GSL formation a balanced N and S supply is required as represented by a species-specific optimal N:S ratio.[8,9] Chen et al. [10] and Krumbein et. al [7] reported that the total GSL concentration in pakchoi and broccoli was enhanced at low N supply. In cabbage, total GSLs were increased by high S supply and low N rates.[11] Increasing N supply decreased seed GSL concentration of oilseed rape when S was deficient, but increased it when S was applied.[12] Schonhof et al. [9] reported that total GSL concentration in broccoli florets was high at insufficient N supply, independent of S supply, and low at insufficient S supply in combination with an optimal N supply. In contrast, a recent study has shown that GSL concentration in broccoli increased by an increased N supply both at low and high S, but it did not respond to N applications above 250 kg ha^{-1}.[13]

To satisfy the increasing health and environmental awareness of consumers, the demand for vegetables with high amounts of health-promoting phytochemicals produced by sustainable production methods needs to be fulfilled.[14] Efficient utilization of available growth resources is fundamental in achieving sustainable systems of agricultural production. For the production of glucosinolate-enriched raw plant material for functional foods or supplements, intercropping could be used as an alternative strategy to mineral fertilization and conventional breeding approaches, strategies which have been used so far.[4] There is a resurgence of interest in intercropping because it may increase the efficient use of natural resources, reduce weed competition, suppress diseases and soil erosion, and prevent nutrients from leaching into deeper soil layers and ground waters, all being significant factors in soil environmental protection and hence affecting plant metabolite formation.[15] Intercropping could be used as an alternative strategy to manipulate N and S balance and hence increase GSLs in *Brassicas*. Nitrogen concentration tended to decrease in cauliflower and cabbage when intercropped with lettuce.[16,17] In this study lettuce was selected to be intercropped with *Brassicas* because it does not have a high S demand, but requires adequate N. Sulfur concentrations in lettuce grown with less

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than 4 mM was approximately 1 mg S g^{-1} of dry matter.[18] In contrast, *Brassicas* have a high S demand and 3−3.5 mg S g^{-1} dry matter is the critical S concentration where visible S deficiency occurs in *Brassica napus*.[1] Moreover, lettuce has similar root characteristics and root depth penetration as a number of *Brassica* species.[19,20] The aim of the study was to evaluate the effect of intercropping with lettuce on glucosinolate concentrations on *Brassicas*. The availability of S will increase relative to N for the *Brassicas*, and this change of the N to S balance in the nutrition of *Brassicas* will enhance their glucosinolate concentration. In addition different N and S supply rates were used in order to examine the impact of lettuce presence in the system.

MATERIALS AND METHODS

Chemicals. The allyl GSL sinigrin was purchased from Sigma-Aldrich (Taufkirchen, Germany). Methanol was obtained from VWR International (Dresden, Germany). Acetonitrile and aryl sulfatase were purchased from Th. Geyer GmbH (Berlin, Germany). All other reagents and solvents used in this study were of HPLC or analytical grade quality.

The Field Experiment. A field experiment was conducted at the Department of Food Science, Aarhus University, Aarslev, Denmark (10°27′E, 55°18′N) on an Agrudalf soil. The upper 0.25 m contains 13% clay, 15% silt, 70% sand, and 1.7% carbon (C). The 0.25−0.50 m layer contain 17% clay, 13% silt, 69% sand, and 0.8% C, and the 0.50−1.0 m layer 19.5% clay, 13% silt, 67% sand, and 0.3% C. The pH_{CaCl_2} ($CaCl_2$ 0.01 M) is 7.1, 6.8, and 6.4 in the 0−0.25, 0.25−0.50, and 0.50−1.0 m soil layers, respectively. During the experimental period, rainfall and air temperature were recorded daily at a meteorological station at the experimental site. Average air daily temperature and precipitation during the growth season are shown in Figure 1. Mean annual precipitation at the site was 624 mm and mean annual air temperature was 7.8 °C.

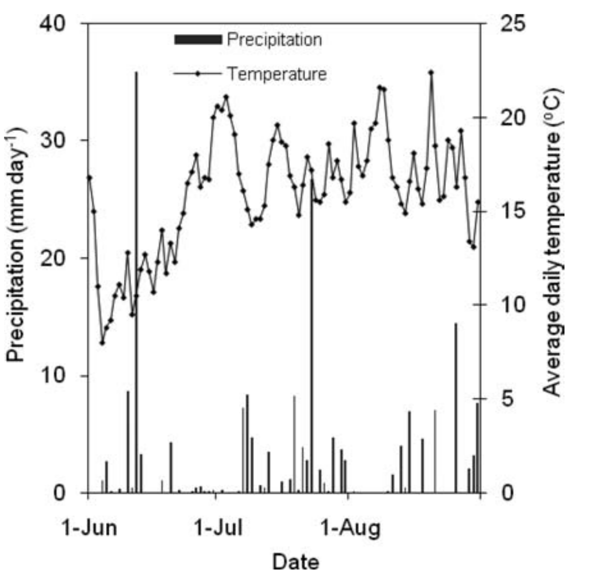

Figure 1. Average daily temperature (line) and precipitation (bars) during the experimental season (June−September, 2009).

The experimental design was a randomized complete block design with three replications. Each block consisted of 12 plots combination of 3 cropping systems and 4 fertilization treatments. The broccoli (*Brassica oleracea* L. var. *italica* cv. Tinman) was intercropped with iceberg lettuce (*Lactuca sativa* L. var. *capitata* cv. Dimantinas RZ). Both crops were grown also in pure stands. Each plot was 1.6 × 3 m, distance between rows was 0.35 m, and within rows was 0.3 m for both intercropping and sole cropping. The intercrop design was based on the replacement principle, with mixed broccoli and lettuce transplant in the same rows. Broccoli seeds were sown on 26 May, 2009, and lettuce on 2 June, 2009, and grown in a greenhouse until transplanting. Both crops were transplanted on 19 June. The four fertilizer treatments were 90 kg ha^{-1} N + 0 kg ha^{-1} S ($N_{90}S_0$), 220 kg ha^{-1} N + 0 kg ha^{-1} S ($N_{220}S_0$), 90 kg ha^{-1} N + 40 kg ha^{-1} S ($N_{90}S_{40}$), 220 kg ha^{-1} N + 40 kg ha^{-1} S ($N_{220}S_{40}$). Urea [$(NH_2)_2CO$, 46−0−0] was used as the N source and Kieserite ($MgSO_4$, 25% MgO and 50% SO_3) was used as the S source. Nitrogen and S fertilizers were broadcast manually on the soil surface 2 days after transplanting.

The plots were kept weed free by repeated manual weeding. Crops were irrigated after the transplanting and the fertilization application, and thereafter irrigation was applied when needed to avoid water stress. During the experimental period, crops received 40 mm irrigation water. Irrigation water was applied via a moveable irrigation boom. The sulfate and nitrate concentrations of the irrigation water were about 32 mg L^{-1} and 3 mg L^{-1}, respectively.

Root Measurements. Root growth of the crops was determined in the pure stands by using minirhizotrons with a diameter of 70 mm and a total length of 1.5 m installed at an angle of 30° from the vertical.[21] In each plot, two minirhizotrons were installed in the inter-row area. Roots were observed by lowering a minivideo camera into the minirhizotrons and recording visible roots on the minirhizotron surface. Root intensity was recorded every two weeks starting four weeks after transplanting by counting the number of roots crossing lines painted on the minirhizotron surface. For every 40 mm along each of two 40 mm wide counting grids on the "upper" surface of each minirhizotron, the number of roots crossing 40 mm of vertical line and 40 mm of horizontal line were counted. As the angle was 30° from the vertical, 40 mm along the minirhizotron surface represented a soil layer of 34.6 mm. From these counts, root intensity was calculated as the number of root intersections m^{-1} line in each soil layer.

Harvest and Sample Preparation. Crops were harvested 50 days after transplanting. Plants were stored at 2 °C for one week. Broccoli and lettuce were separated into edible part (broccoli florets and lettuce heads) and crop residues (remaining stem and leaves). To determine dry matter (DM) content, two samples per treatment were placed at 80 °C in a forced air-drying oven for 20 h. The DM samples were then used for N and S analysis. For glucosinolate analysis, samples of five broccoli florets from each plot were used. The florets were cut, immediately frozen (−40 °C), freeze-dried, and ground.

Initial soil mineral N and S were determined in April before the establishment of the experiment. After harvest, soil samples (nine replicates per plot) were analyzed for N and S concentration in all treatments. Soil samples were taken randomly with a pistol auger (inner diameter 14 mm). In April, samples were taken of the soil layers 0−0.25 m, 0.25−0.50 m, and 0.50−0.75 m, and in August 0−0.25 m, 0.25−0.50 m, and 0.50−1.0 m. The soil samples were frozen at −18 °C within 24 h from sampling.

The Pot Experiment. A pot experiment was carried out from 8 May to 8 June, 2010, in a greenhouse at the Department of Food Science, Aarhus University, Aarslev, in order to eliminate the above-ground competition which occurred in the field experiment. The soil used was collected from the top 15 cm of a field located at the department. The soil was air-dried, sieved (<5 mm), and mixed with sand (2:1) to ensure good porosity for air and water. The mixture of soil was placed in 7 L plastic pots (upper diameter = 0.25 m, height = 0.20 m).

The red leaf mustard (*Brassica juncea* L. cv Red Giant) was intercropped with leaf lettuce (*Lactuca sativa* L. var. *capitata* cv. Lugano RZ). The cultivars were selected because they were both fast growing. In the intercropping treatment, one side of each pot was planted with one red leaf mustard seedling and the other side with one lettuce seedling. Both crops were grown also in pure stands with two plants per pot. In order to minimize the aerial interaction and competition between crops, plants were separated by a Polystyrene foam board both in intercropping and sole cropping treatments. Two levels of N were supplied in the form of urea at 0.9 g pot^{-1} and 2 g pot^{-1} corresponding to 203 and 406 kg N ha^{-1}, respectively. Sulfur

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was applied at two different rates of 44 and 88 kg ha^{-1} in the form of Kieserite corresponding to 0.2 and 0.45 g per pot. In the pot experiment double the amount of fertilizers was used compared to the field experiment in order to ensure sufficient plant growth.

The pots were arranged on a greenhouse bench in a complete randomized block design with four replicates of each of the 12 treatments. The average day and night greenhouse temperatures were 20 and 14 °C, respectively; the average day length during the experiment was 13 h. The pots were watered daily with precollected rainwater as needed to avoid water stress. To avoid leaching losses from the pots, a drainage tray was placed under each pot. Any leachate collected in the trays was reapplied to the pot. Plants were harvested 31 days after transplanting into the pots. The main midrib from the red leaf mustard leaves was removed prior to the analysis because it contains low GSL concentration and could lead to a bias within the leaf sample. The samples of leaf mustard were immediately deep frozen (−40 °C), then freeze-dried, ground, and analyzed for GSLs, N, and S concentrations. The lettuce samples were oven-dried at 80 °C for 20 h, ground, and analyzed for N and S concentrations. The dry matter yield was recorded.

Glucosinolate Analysis. A modified HPLC method reported by Krumbein et al.[22] was used to determine the desulfo-glucosinolate profiles. Duplicates of freeze-dried sample material (0.02 g) were heated to and incubated at 75 °C for 1 min, and then extracted with 0.75 mL of 70% methanol. The extracts were heated for 10 min at 75 °C and then, after adding 0.2 mL 0.4 M barium acetate, centrifuged at 4000 rpm for 5 min. The supernatants were removed, and the pellets were extracted twice more with 0.5 mL of 70% methanol (70 °C), shaken vigorously in a Vortex mixer to dissolve pellets, and centrifuged. Just prior the first extraction 100 μL of a 0.5 M stock solution of sinigrin (2-propenyl GSL) in methanol was added to one of the duplicated as internal standard. The supernatants were combined and applied to a 250 μL DEA-Sephadex A-25 ion-exchanger (acetic acid-activated, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and washed with bidistilled water. After the application of 100 μL of purified aryl sulfates solution and 12 h incubation, desulfo-compounds were eluted with 1.5 mL of double-distilled water.

Desulfo-glucosinolate analysis was carried out by HPLC (Merck HPLC pump L-7100, DAD detector L-7455, automatic sampler AS-7200 and HPLC Manager-Software D-7000) using Spherisorb ODS2 column (3 μm, 125 × 4 mm). A gradient of 0−20% acetonitrile in water selected from 2 to 34 min, followed by 20% acetonitrile in water until 40 min, and then 100% acetonitrile for 10 min. The determination was conducted at a flow of 0.7 mL min^{-1} and a wavelength of 229 nm. Glucosinolate concentrations were calculated using sinigrin as internal and external standard and the response factor of each compound relative to sinigrin (Official Journal of the European Communities, 1990, *L 170*, 28−34). The well-known desulfo-glucosinolates were identified according to previous work[23] from the protonated molecular ions $[M + H]^+$ and the fragment ions corresponded to $[M + H - \text{glucose}]^+$ by HPLC-ESI−MS^2 using Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) in the positive ionization mode. Determinations of desulfo-glucosinolates were performed in duplicate. The desulfo-glucosinolates determinate are shown in Table 1.

N and S Analysis. In the field experiment, N and S concentrations were measured both in the edible parts and the crop residues. Total plant N was determined after dry oxidation by the Dumas method (Elementar Vario EL, Hanau, Germany) and total sulfur was determined by using NDIR (nondispersive infrared gas analysis) optic to detect the sulfur dioxide formed. Finely ground samples were weighed into quartz boats and delivered into the hot zone of a multi EA 2000 CS (Analytic Jena AG, Jena, Germany). Then the samples were pyrolyzed and oxidized at 1300 °C in a stream of oxygen (99.5%). Both measurements were performed in duplicate.

For analysis of soil inorganic N, 100 g of soil was shaken vertically for 1 h with 200 mL of KCl solution (1 M). The soil extract was filtered and analyzed for NH_4^+ and NO_3^- concentration by continuous flow analysis (CFA). Total inorganic sulfate was extracted by shaking soil (40 g) with 400 mL of $CaCl_2^-$ solution (0.0125 M) for an hour.

Table 1. Glucosinolates (GSL) Determinate by HPLC and Their Nomenclature

GSL type	common name	semi systematic name	occurrence
aliphatic			
alkyl	glucoiberin	3-methysulfinylpropyl	broccoli
	glucoraphanin	4-methylsulfinylbutyl	broccoli
alkenyl	sinigrin	2-propenyl	red leaf mustard
	gluconapin	3-butenyl	red leaf mustard
hydroxyl alkenyl	progoitrin	2-hydroxy-3-butenyl	red leaf mustard
indole			
	glucobrassicin	3-indolylmethyl	broccoli, red leaf mustard
	neoglucobrassicin	1-methoxy-3-indolylmethyl	broccoli
	4-methoxy-glucobrassicin	4-methoxy-3-indolylmethyl	broccoli, red leaf mustard

Extracts were filtered and sulfate was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES).

Data Analysis. The productivity of the intercropping was evaluated by the land equivalent ratio (LER). The LER provides a comparative measure of the biological efficiency of pure and mixed species cropping systems.[24] Land equivalent ratio for an intercrop system is the sum of the partial LER values for crop A (L_A) and B (L_B) in accordance with

$$L_A = \frac{Y_{IA}}{Y_{SA}}, \; L_B = \frac{Y_{IB}}{Y_{SB}}$$

$$\text{LER} = L_A + L_B$$

For the DM-based LER values, Y_{IA} and Y_{IB} refer to the edible product DM production of the crops A and B in intercropping, and Y_{SA} and Y_{SB} are the edible product DM production of crops A and B in sole cropping. For N- and S-based LER values, Y refers to N and S uptake by the edible parts of the two crops. Nutrient uptake (kg ha^{-1} or g pot^{-1}) of individual plant species was calculated by multiplying above ground nutrient content (mg g^{-1} DM) and biomass (kg ha^{-1} or g pot^{-1}). Whereas an LER greater than one (LER > 1) indicates an advantage from intercropping in terms of the use of environmental resources for plant growth, when LER < 1, resources are used more efficiently by sole crops than by intercrops.

Statistical Analysis. Three-way analysis of variance of the effects of N supply, S supply, and cropping system with their interactions on tested parameters was conducted to separate the sources of variation using the GLM procedure of the SAS statistical package (SAS Institute Inc., Cary, NC, USA, 1990). Variables were log-transformed if the assumptions of normality or homogeneity of variances were not met. The significance level was set at p = 0.05. In the pot experiment, flowering mustard plants with GSL concentrations significantly different from GSL concentrations in the nonflowering plants were excluded from the statistical analysis.

■ RESULTS

The Field Experiment. *Soil N and S.* Increased N supply increased soil N concentration after sole cropping of lettuce (Table 2). In intercropping and sole cropping of broccoli, this effect was smaller and only significant when S fertilizer was not applied together with the N fertilizer. Soil N concentration did not differ between intercropping and sole cropping of broccoli, but they were higher in sole cropping of lettuce.

In the soils where S fertilization was applied, the S concentration was higher than in the unamended soils (Table 2). No differences were found in soil S after intercropping and sole cropping of broccoli, but higher soil S was found after sole cropping of lettuce where S had been applied.

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Table 2. Effects of Cropping System and Fertilization Treatments on N and S Concentrations ($kg\ ha^{-1}$) in the Soil of the Field Experiment at Harvest[a]

cropping system	fertilization	inorganic N (0−1.0 m)	inorganic S (0−1.0 m)
intercrop	$N_{90}S_0$	38 ± 9	37 ± 11
	$N_{90}S_{40}$	37 ± 4	57 ± 19
	$N_{220}S_0$	52 ± 11	37 ± 6
	$N_{220}S_{40}$	41 ± 7	45 ± 5
sole lettuce crop	$N_{90}S_0$	47 ± 11	36 ± 7
	$N_{90}S_{40}$	51 ± 5	82 ± 14
	$N_{220}S_0$	65 ± 0	40 ± 3
	$N_{220}S_{40}$	64 ± 1	85 ± 4
sole broccoli crop	$N_{90}S_0$	34 ± 2	37 ± 17
	$N_{90}S_{40}$	38 ± 7	50 ± 6
	$N_{220}S_0$	48 ± 9	29 ± 4
	$N_{220}S_{40}$	42 ± 6	51 ± 4
significance[b]			
	N	***	NS
	S	NS	***
	C	***	***
	N × C	NS	NS
	S × C	NS	**
	N × S	NS	NS
	N × S × C	NS	NS

[a]Where N: nitrogen; S: sulfur; C: cropping system. [b]Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean ± standard deviation (SD) of three replicates.

Table 3. Effects of Cropping System and Fertilization Treatments on the Edible Plant Part and Total Above Ground Dry Matter Production ($kg\ ha^{-1}$) in the Field Experiment[a]

		broccoli		lettuce	
cropping system	fertilization	edible	total	edible	total
intercrop	$N_{90}S_0$	689 ± 150	4206 ± 273	314 ± 75	450 ± 88
	$N_{90}S_{40}$	663 ± 123	4454 ± 245	266 ± 17	383 ± 26
	$N_{220}S_0$	726 ± 221	4558 ± 54	194 ± 49	294 ± 85
	$N_{220}S_{40}$	819 ± 136	5000 ± 202	162 ± 24	232 ± 37
sole crop	$N_{90}S_0$	567 ± 123	5188 ± 201	2536 ± 205	3658 ± 241
	$N_{90}S_{40}$	573 ± 54	5097 ± 251	2484 ± 139	3502 ± 116
	$N_{220}S_0$	742 ± 132	5433 ± 373	2337 ± 90	3538 ± 178
	$N_{220}S_{40}$	774 ± 126	5761 ± 423	2318 ± 201	3486 ± 199
significance[b]					
	N	NS	***	**	NS
	S	NS	NS	NS	NS
	C	NS	***	***	***
	N × C	NS	NS	NS	NS
	S × C	NS	NS	NS	NS
	N × S	NS	NS	NS	NS
	N × S × C	NS	NS	NS	NS

[a]Where N: nitrogen; S: sulfur; C: cropping system. [b]Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean (with SD) of three replicates.

Above Ground Biomass Production. Intercropping decreased the total above ground biomass production of both broccoli and lettuce (Table 3). The reduction in biomass due to intercropping was greater in lettuce which was reduced up to 93%. Increasing the N supply from 90 to 220 $kg\ ha^{-1}$ increased broccoli above ground biomass but had no effect on lettuce biomass. Sulfur fertilization did not influence broccoli or lettuce above ground production. In contrast to total above ground biomass production of broccoli, intercropping and fertilization treatments did not influence florets biomass (edible plant parts) production of broccoli.

Calculated LER based on the total above-ground DM production (Figure 2a) was lower than 1.0 in all fertilization treatments. Partial LER values of broccoli were greater than 0.8, while the LER values of lettuce varied between 0.07 to 0.12. Fertilization treatments did not affect the LER value or the partial LERs.

Root Growth. The two vegetables had different root characteristics (Figure 3). Lettuce and broccoli showed quite similar rates of rooting depth penetration, but the root intensity and root distribution in the soil varied between the crops. The root intensity, four weeks after transplanting, was comparable in both crops, and they both showed the highest root intensity in

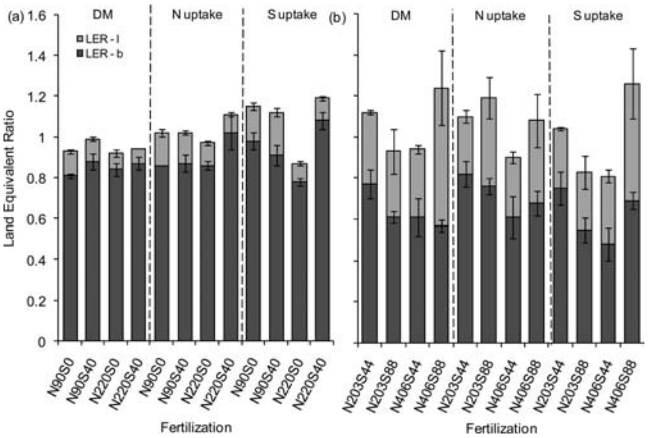

Figure 2. Land equivalent ratio (LER) based on total dry matter (DM) production, N and S uptake on the field (a) and pot (b) experiments. Bars represent the standard errors (where $n = 3$ for the field experiment and $n = 4$ for the greenhouse experiment). LER - l: Partial LER of lettuce; LER - b: Partial LER of broccoli (a) and red leaf mustard (b).

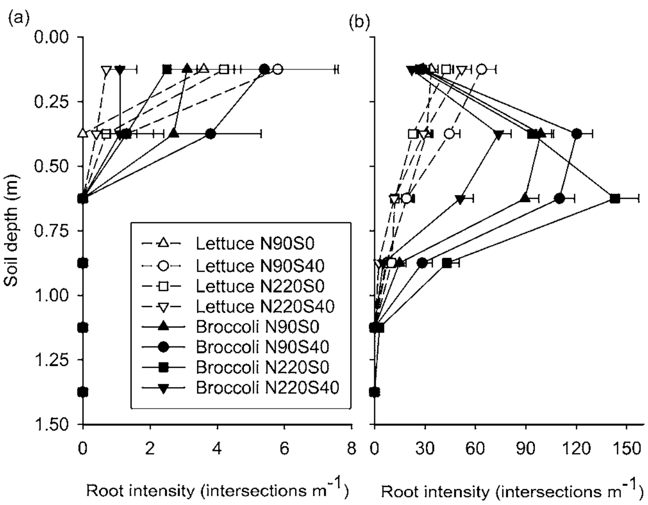

Figure 3. Average root intensity in the 0−1.5 m soil profile in the field experiment four weeks after transplanting (a) and two weeks before harvest (b). Where N: nitrogen; S: sulfur. Bars represent the standard errors (where $n = 2$).

the top 0.25 m soil layer (Figure 3a). However, broccoli had established higher root intensity than lettuce in the soil layer between 0.25 and 0.5 m. Fertilization affected root growth of the two crops; higher root intensity was observed when low N and high S were applied (Figure 3b). At the final measurement (2 weeks before harvest), the root intensity of broccoli was much higher than that of lettuce, but in the top 0.25 m layer lettuce had the highest root intensity. Below this, the root intensity of lettuce declined gradually, whereas broccoli had its highest root intensity in the 0.25 and 0.75 m soil layer. Between 0.75 and 1 m, broccoli still had higher root intensity than lettuce, as lettuce showed practically no roots in this soil layer.

N and S Accumulation. Nitrogen and S accumulation in broccoli and lettuce are shown in Table 4. Intercropping affected N concentrations in broccoli florets and total N uptake by broccoli. When broccoli was intercropped with lettuce it had lower floret N concentrations and lower N uptake compared to sole cropping of broccoli. Nitrogen concentrations and N uptake in all examined organs of broccoli responded to N supply, but these were unaffected by S level and no significant interactions were found. The effect of N application on N concentration was stronger in the broccoli crop residues than in the edible part. Increasing the N supply increased N concentration in broccoli florets by 15−19%, while in broccoli residues N concentration increased by 31−62%.

Fertilization with S increased total S uptake and the S concentration both in broccoli florets and residues, at both N fertilization levels (Table 4). The response of S concentration to S fertilization was greater in broccoli residues than in florets. Increasing the N supply from 90 to 220 kg ha^{-1} had no effect on tissue S concentrations in broccoli florets or residues. However, increasing the N supply at the low S rate decreased S uptake by broccoli while in the high S rate increased S uptake. Intercropping only affected S concentrations of broccoli residues under the high N and S treatment, with a 28% increase in S concentration observed in intercropping compared to sole cropping of broccoli. A significant N supply × S supply × cropping system interaction for S accumulation in broccoli residues was observed.

Nitrogen and S uptake by lettuce was reduced by intercropping, as a result of the limited lettuce growth (Table 4). Nitrogen fertilization enhanced N and S uptake by lettuce only in the sole cropping treatment.

All the LER values for N and S uptake except from the $N_{220}S_0$ were above 1.0 and varied between 1.02 to 1.19 (Figure 2a). The LER values based on total N and S accumulation indicated that in general N and S were used 2−11% and 12−19% more efficiently in intercrops than sole crops. Fertilization treatments influenced only the LER value based on total S uptake; at the high N level the LER increased by increasing the S level. Partial LER values of broccoli based on N uptake were 5−11 times higher than lettuce and LER values based on S uptake were 4−10 times higher.

Glucosinolates. Five individual GSLs, namely, the aliphatic GSLs glucoraphanin and glucoiberin and the indole GSLs glucobrassicin, neoglucobrassicin, and 4-methoxy-glucobrassicin were quantitatively determined in broccoli florets (Table 1). The total GSL was calculated as the sum of the individual GSLs.

The highest total GSL level (4137 μg g^{-1} DM) was obtained at high N and S supply in sole cropped broccoli, while the lowest level (2458 μg g^{-1} DM) was observed in intercropped broccoli at $N_{220}S_0$ (Table 5). Total GSL concentrations increased by S fertilization, whereas N fertilization reduced GSL concentrations when N was added without S. Total and aliphatic GSL concentrations were not affected by intercropping (Table 5).

Total aliphatic GSL concentration responded to S application only under high N availability. Sulfur fertilizer increased total aliphatic GSLs by up to 51% in intercropping and by up to 49% in sole cropping. Without simultaneous S fertilization, N fertilization decreased aliphatic GSLs by up to 45% and 34% in inter- and sole cropping, respectively.

A negative correlation ($p < 0.01$) between aliphatic GSLs and N concentration was shown in the regression analysis (Figure 4a). When S concentration in broccoli florets was higher than 6.0 mg g^{-1} DM, aliphatic GSLs were between 1.5 and 2.0 mg g^{-1} DM, but these reached 1.0 mg g^{-1} DM when S concentrations were lower 6.0 mg g^{-1} DM (Figure 4b). Consequently, a significant negative correlation ($p < 0.001$) between aliphatic GSLs and the N:S ratio was determined in the regression analysis (Figure 4c).

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Table 4. Effects of Cropping System and Fertilization Treatments on N and S Concentration (mg g^{-1} DM) and Total N and S Uptake (kg ha^{-1}) in Broccoli and Total N and S Uptake (kg ha^{-1}) by Lettuce in the Field Experiment[a]

		nitrogen				sulfur			
		broccoli				broccoli			
		concentration			lettuce	concentration			lettuce
cropping system	fertilization	florets	residues	total uptake	total uptake	florets	residues	total uptake	total uptake
intercrop	$N_{90}S_0$	32 ± 2	20 ± 1	93 ± 4	16 ± 3	6 ± 1	5 ± 0	20 ± 3	1 ± 0
	$N_{90}S_{40}$	32 ± 3	19 ± 2	93 ± 6	14 ± 1	7 ± 1	8 ± 1	33 ± 2	1 ± 0
	$N_{220}S_0$	39 ± 3	29 ± 4	141 ± 13	12 ± 2	5 ± 1	3 ± 0	16 ± 1	1 ± 0
	$N_{220}S_{40}$	38 ± 2	31 ± 3	158 ± 7	11 ± 1	7 ± 0	8 ± 0	41 ± 2	1 ± 0
sole crop	$N_{90}S_0$	35 ± 0	19 ± 1	109 ± 6	96 ± 15	6 ± 1	4 ± 0	21 ± 2	6 ± 0
	$N_{90}S_{40}$	36 ± 1	19 ± 1	107 ± 8	89 ± 6	8 ± 0	7 ± 1	36 ± 4	6 ± 1
	$N_{220}S_0$	41 ± 5	28 ± 4	162 ± 10	118 ± 5	6 ± 1	3 ± 1	20 ± 2	8 ± 1
	$N_{220}S_{40}$	41 ± 1	25 ± 1	156 ± 15	119 ± 4	7 ± 1	6 ± 1	37 ± 2	7 ± 1
significance[b]									
	N	***	***	***	**	NS	NS	NS	NS
	S	NS	NS	NS	NS	***	***	***	NS
	C	**	NS	**	***	NS	*	NS	***
	N × C	NS	NS	NS	***	NS	NS	NS	**
	S × C	NS	NS	NS	NS	NS	NS	NS	NS
	N × S	NS	NS	NS	NS	NS	NS	*	NS
	N × S × C	NS	NS	NS	NS	NS	*	NS	NS

[a]Where N: nitrogen; S: sulfur; C: cropping system. [b] Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean ± standard deviation (SD) of three replicates.

Table 5. Effects of Cropping System and Fertilization Treatments on Glucosinolate Concentration (μg g^{-1} DM) and N:S Ratio in Broccoli Florets of the Field Experiment[a]

			glucosinolate[b]							
cropping system	fertilization	N:S ratio	GRA	GIB	GBS	NGB	MGB	total GSLs	total aliphatic GSLs	total indole GSLs
intercrop	$N_{90}S_0$	5 ± 0	1458 ± 249	446 ± 58	252 ± 9	1246 ± 345	32 ± 6	3433 ± 242	1904 ± 307	1529 ± 354
	$N_{90}S_{40}$	5 ± 0	1425 ± 144	434 ± 48	275 ± 27	1661 ± 440	30 ± 4	3824 ± 280	1859 ± 191	1966 ± 471
	$N_{220}S_0$	8 ± 0	777 ± 90	278 ± 17	229 ± 33	1145 ± 157	29 ± 2	2458 ± 222	1055 ± 104	1403 ± 149
	$N_{220}S_{40}$	5 ± 0	1221 ± 125	370 ± 23	327 ± 34	1580 ± 208	34 ± 5	3533 ± 386	1591 ± 148	1942 ± 240
sole crop	$N_{90}S_0$	6 ± 1	1210 ± 80	430 ± 15	263 ± 59	1510 ± 163	31 ± 2	3443 ± 318	1639 ± 95	1803 ± 224
	$N_{90}S_{40}$	5 ± 0	1359 ± 199	445 ± 78	277 ± 45	1754 ± 319	35 ± 5	3869 ± 493	1803 ± 275	2066 ± 368
	$N_{220}S_0$	7 ± 1	774 ± 14	315 ± 28	260 ± 92	1561 ± 535	32 ± 7	2941 ± 592	1089 ± 41	1852 ± 631
	$N_{220}S_{40}$	6 ± 0	1197 ± 46	427 ± 22	342 ± 9	2130 ± 223	40 ± 2	4137 ± 280	1625 ± 63	2513 ± 218
significance[c]										
	N	***	***	***	NS	NS	NS	*	***	NS
	S	***	**	**	NS	**	NS	***	**	**
	C	NS	NS	NS	NS	*	NS	NS	NS	*
	N × C	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S × C	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N × S	*	**	**	NS	NS	NS	*	**	NS
	N × S × C	*	NS	NS	NS	NS	NS	NS	NS	NS

[a]Where N: nitrogen; S: sulfur; C: cropping system. [b]GRA: glucoraphanin; GIB: glucoiberin; GBS: glucobrassicin; NGB: neoglucobrassicin; MGB: 4-methoxy-glucobrassicin; GSLs: glucosinolates. [c]Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean ± standard deviation (SD) of three replicates.

Changes in the total indole GSLs were mainly due to variations in neoglucobrassicin, and comparable low concentrations of glucobrassicin and 4-methoxy-glucobrassicin were detected (Table 5). Sulfur fertilization increased neoglucobrassicin concentrations, regardless of N supply. Neoglucobrassicin concentration in broccoli florets was reduced in intercropping as compared to sole cropping especially at high N and S supply.

In contrast to aliphatic GSLs, a slight positive correlation ($p < 0.05$) between indole GSLs and N concentration in broccoli florets was found (Figure 5a). The correlation ($p < 0.001$) between indole GSLs and S concentration in broccoli florets (Figure 5b) was stronger than the correlation ($p < 0.05$) of the aliphatic GSLs (Figure 4b). With S concentrations above 7.0 mg g^{-1} DM total indole GSLs were around 2.0 mg g^{-1} DM on average, but they decreased to ca. 1.5 mg g^{-1} DM with S concentrations below 7.0 mg g^{-1} DM. In contrast to aliphatic GSLs, the correlation ($p < 0.05$) of indole GSLs and N:S ratio was weak (Figure 5c).

The Pot Experiment. *Dry Matter Production.* Red leaf mustard above ground biomass was influenced by intercropping but remained unaffected by N and S fertilization or any

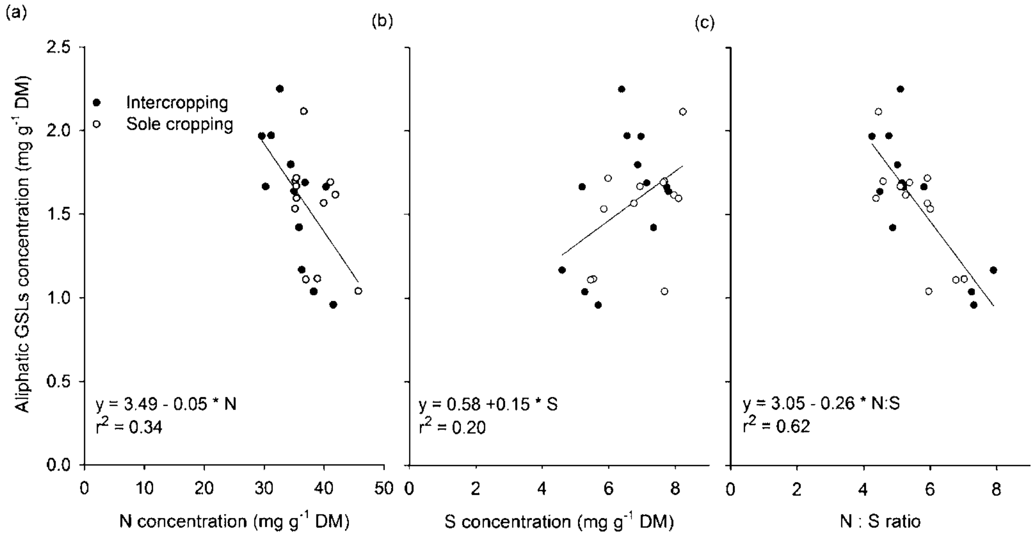

Figure 4. Influence of N (a) and S (b) concentration and N:S ratio (c) on total aliphatic glucosinolate (GSL) concentration in broccoli in the field experiment.

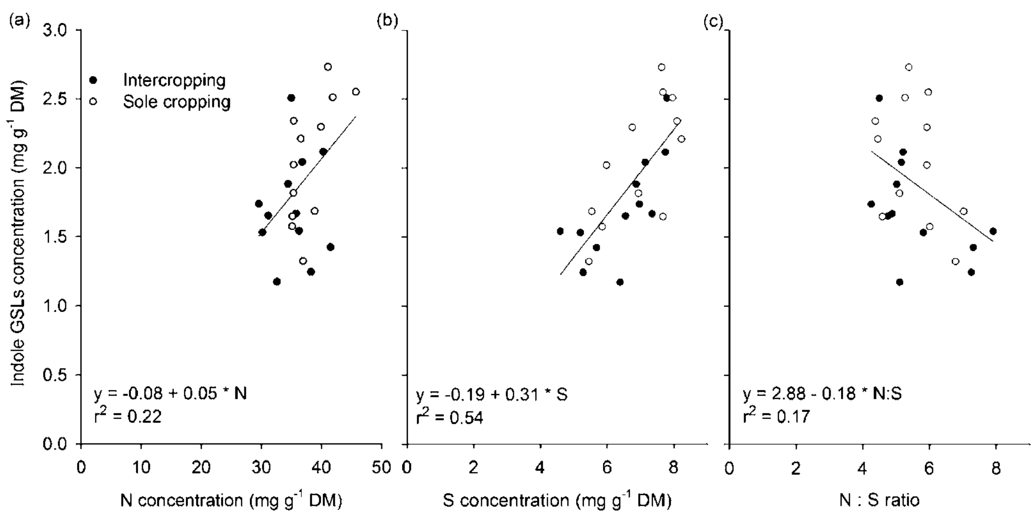

Figure 5. Influence of N (a) and S (b) concentration and N:S ratio (c) on total indole glucosinolate (GSL) concentration in broccoli in the field experiment.

interaction (Table 6). When red leaf mustard was intercropped with lettuce DM production was 1.1−1.5 times higher compared to sole cropping. Lettuce above ground biomass production was neither affected by intercropping nor fertilization (Table 6). Red leaf mustard DM was 3.9−7.8 times higher than that of lettuce.

Intercropping of red leaf mustard and lettuce showed an advantage (LER > 1) in terms of total yields only at the $N_{203}S_{44}$ and $N_{406}S_{88}$ (Figure 2b). The partial LER values of the red leaf mustard varied between 0.61 and 0.77 and the lettuce partial LER between 0.32 and 0.67. The fertilization treatments did not influence the LER values based on DM production.

N and S Accumulation. Nitrogen concentrations in red leaf mustard were affected by the cropping system, the fertilization treatments, and the S supply × cropping system interaction (Table 6). Increasing N supply from 203 to 406 kg ha^{-1} increased the N concentrations in mustard leaves by 15−33%. Enhanced S fertilization increased N concentration in red leaf mustard when it was grown with lettuce. In intercropping, N concentrations in mustard leaves were higher than in sole cropping. In contrast, intercropping decreased the N concentrations in lettuce (Table 6). Moreover, lettuce N concentrations were affected by the N supply × S supply, N supply × cropping system, and N supply × S supply × cropping system interactions. Compared to N, the concentration of S in leaves of mustard was much less affected and only the impact of S supply was significant (Table 6). Sulfur concentrations in lettuce leaves were unaffected by the cropping system or the fertilization treatments.

The LER value based on total N uptake varied between 0.9 and 1.19 and on total S uptake between 0.81 and 1.26 (Figure 2b). The LER values based on N and S uptake were unaffected by the fertilization treatments. The partial LER values of red leaf mustard based on N and S was higher than the partial LER values of lettuce.

Glucosinolates. Individual glucosinolates, namely, the aliphatic GSLs progoitrin, gluconapin, and sinigrin, and the indole GSLs glucobrassicin and 4-methoxy-glucobrassicin were determined in red leaf mustard (Table 7). Aliphatic GSLs were the major fraction of total GSLs in red leaf mustard at 98−99%.

Table 6. Effects of Cropping System and Fertilization Treatments on above Ground Dry Matter Production (g plant^{-1}), N and S Concentration (mg g^{-1} DM) and Uptake (mg pot^{-1}) in Red Leaf Mustard and Lettuce in the Pot Experiment[a]

		red leaf mustard					lettuce				
			concentration		total uptake			concentration		total uptake	
cropping system	fertilization	dm yield	nitrogen	sulfur	nitrogen	sulfur	DM yield	nitrogen	sulfur	nitrogen	sulfur
intercrop	$N_{203}S_{44}$	10 ± 1	43 ± 3	6 ± 1	436 ± 52	57 ± 11	1 ± 0	30 ± 3	2 ± 0	38 ± 6	2 ± 1
	$N_{203}S_{88}$	9 ± 2	47 ± 1	6 ± 0	432 ± 79	59 ± 15	1 ± 0	36 ± 8	2 ± 0	45 ± 22	2 ± 1
	$N_{406}S_{44}$	10 ± 2	48 ± 1	5 ± 1	443 ± 81	54 ± 20	1 ± 0	38 ± 3	2 ± 0	52 ± 9	3 ± 1
	$N_{406}S_{88}$	10 ± 1	56 ± 2	8 ± 1	561 ± 42	74 ± 6	2 ± 1	26 ± 2	2 ± 0	49 ± 20	3 ± 1
sole crop	$N_{203}S_{44}$	7 ± 1	40 ± 4	6 ± 1	538 ± 57	79 ± 25	2 ± 0	39 ± 5	2 ± 0	141 ± 27	9 ± 2
	$N_{203}S_{88}$	8 ± 1	38 ± 4	7 ± 0	568 ± 50	107 ± 19	2 ± 1	37 ± 2	2 ± 0	128 ± 28	8 ± 2
	$N_{406}S_{44}$	8 ± 1	49 ± 5	5 ± 1	823 ± 179	97 ± 11	2 ± 0	43 ± 4	2 ± 0	178 ± 31	9 ± 3
	$N_{406}S_{88}$	9 ± 1	50 ± 6	6 ± 0	864 ± 82	109 ± 9	1 ± 0	45 ± 1	2 ± 0	130 ± 11	6 ± 0
significance[b]											
	N	NS	***	NS	***	NS	NS	NS	NS	NS	NS
	S	NS	*	***	NS	**	NS	NS	NS	NS	NS
	C	**	**	NS	***	***	NS	***	NS	***	***
	N × C	NS	NS	NS	**	NS	NS	*	NS	NS	NS
	S × C	NS	*	NS	NS	NS	NS	NS	NS	*	NS
	N × S	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
	N × S × C	NS	NS	NS	NS	*	NS	***	NS	NS	NS

[a]Where N: nitrogen; S: sulfur; DM: dry matter; C: cropping system. [b]Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean ± standard deviation (SD) of four replicates.

Table 7. Effects of Cropping System and Fertilization Treatments on Glucosinolate Concentration (μg g^{-1} DM) and N:S Ratio in Red Leaf Mustard in the Pot Experiment[a]

			glucosinolate[b]							
cropping system	fertilization	N:S ratio	PRO	SIN	GNA	MGB	GBS	total GSLs	total aliphatic GSLs	total Indole GSLs
intercrop	$N_{203}S_{44}$	7.7 ± 1	5 ± 6	10586 ± 1594	452 ± 72	18 ± 4	98 ± 14	11159 ± 1644	11043 ± 1629	116 ± 17
	$N_{203}S_{88}$	7.4 ± 1	7 ± 12	10335 ± 1333	510 ± 176	18 ± 5	104 ± 26	10974 ± 1494	10852 ± 1504	122 ± 23
	$N_{406}S_{44}$	9.5 ± 3	11 ± 9	8478 ± 1751	327 ± 26	17 ± 7	82 ± 24	8915 ± 1799	8816 ± 1785	100 ± 25
	$N_{406}S_{88}$	7.3 ± 0	16 ± 13	10885 ± 1567	498 ± 122	26 ± 4	145 ± 29	11571 ± 1630	11400 ± 1659	171 ± 32
sole crop	$N_{203}S_{44}$	7.1 ± 1	7 ± 5	8368 ± 1917	364 ± 97	14 ± 4	89 ± 24	8842 ± 2020	8739 ± 2000	103 ± 27
	$N_{203}S_{88}$	5.4 ± 1	7 ± 12	7784 ± 699	364 ± 45	9 ± 2	66 ± 27	8230 ± 767	8155 ± 739	75 ± 29
	$N_{406}S_{44}$	9.2 ± 2	2 ± 4	8218 ± 1831	410 ± 77	16 ± 7	73 ± 18	8720 ± 1911	8630 ± 1908	90 ± 15
	$N_{406}S_{88}$	8.0 ± 1	6 ± 5	10288 ± 1092	458 ± 141	18 ± 7	105 ± 13	10874 ± 1246	10751 ± 1231	123 ± 20
significance[c]										
	N	**	NS	NS	NS	*	NS	NS	NS	NS
	S	*	NS	NS	NS	NS	*	NS	NS	*
	C	NS	NS	*	NS	*	**	*	*	**
	N × C	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S × C	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N × S	NS	NS	*	NS	NS	**	*	*	**
	N × S × C	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Where N: nitrogen; S: sulfur; C: cropping system. [b]PRO: progoitrin; GNA: gluconapin; SIN: sinigrin; GBS: glucobrassicin; GSLs: glucosinolates; MGB: 4-methoxy-glucobrassicin. [c]Levels of significance: NS, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Each data represents the mean ± standard deviation (SD) of four replicates.

The most predominant GSL was sinigrin accounting for 70–98% of total GSLs, followed by gluconapin (3–5% of total GSLs). Total GSL concentrations varied between 8230 and 11571 μg g^{-1} DM with different cropping systems and fertilization supply (Table 7). The highest GSL concentrations were recorded in pots that received 88 kg S ha^{-1} and 406 kg N ha^{-1} irrespective of the cropping system. Sinigrin concentration in red leaf mustard was significantly influenced by intercropping; GSLs were higher in intercropping, independent of the fertilization levels. The significant N supply × S supply interaction indicated that GSL concentration, as a response to S supply, is dependent on N supply. Increasing S supply increased sinigrin concentrations only at the high N level, whereas a slight reduction was observed at the low N level. The results presented here suggest that N can both increase and decrease GSL concentrations depending on the S supply. With low S supply, total GSL concentrations were higher at low N supply. However, at the high S level, increasing N supply increased total GSLs by 5% in the intercropping and 32% in the sole cropping systems. The aliphatic GSLs progoitrin and gluconapin were unaffected by either the intercropping or the fertilization treatments.

Indole GSLs concentrations were generally low in red leaf mustard plants (1–1.5% of the total GSLs) (Table 7).

Intercropping increased indole GSLs up to 63%. In addition S fertilization affected total indole GSLs and glucobrassicin concentrations; in general, increased S fertilization enhanced indole GSLs with the exception of the low N treatment in the intercropping system. A significant interaction was observed between N and S fertilization; increasing N when the S fertilization was high resulted in an increase of indole GSLs. Increasing N supply led to a decrease in indole GSLs when S supply was low.

In contrast to the field experiment, aliphatic GSL concentrations showed a positive significant correlation only with the N concentrations ($r^2 = 0.23$, $p < 0.01$), whereas indole GSLs were positively correlated both with N ($r^2 = 0.48$, $p < 0.001$) and S ($r^2 = 0.20$, $p < 0.05$) concentrations in accordance with the field experiment. Neither aliphatic nor indole GSLs were correlated with the N:S ratio.

■ DISCUSSION

The Field Experiment. Variation in the concentration, as well as in the pattern, of GSL occurred depending on the cropping conditions. In the field experiment total, aliphatic and indole GSLs were within the ranges reported in previous studies.[4] The high concentrations of glucoraphanin and neoglucobrassicin determined in broccoli in this study are consistent with the findings of Baik et al.[5]

Although the present results confirmed that the balance between N and S plays an important role in the regulation of the synthesis and/or accumulation of GSLs, our hypothesis that intercropping will increase GSL concentrations was not verified in the field experiment. The low LER values indicate no yield advantage in the broccoli/lettuce intercropping system. The high partial LER value of broccoli indicated that broccoli was the dominant component in the intercropping. Similarly, broccoli dominated and strongly reduced the crop yields of pea and cabbage during intercropping.[25] The limited growth of the lettuce and its reduced yield were mainly attributed to irradiation competition as broccoli completely shaded the lettuce. The root data showed that lettuce could be able to compete well with broccoli for nutrients, though broccoli showed higher total root growth, lettuce built higher root densities in the topsoil and had approximately the same root depth development as broccoli, in accordance with results of Thorup-Kristensen.[19,20] However, below ground competition possibly also occurred as the root density of broccoli was higher in the lower soil layers than that of lettuce at the end of the cultivation period. Subsequently, total N and S uptake by lettuce was limited and intercropping did not influence the balance of inorganic N and S left in the soil by the crops significantly or the balance between N and S in the broccoli crop.

Glucoraphanin is the main aliphatic GSL found in broccoli florets. Our results agree with those of Schonhof et al.[9] who found that broccoli plants grown with a low N supply showed no significant differences in the concentration of glucoraphanin in response to different S supplies, but this changed when grown with enhanced N. Moreover, they showed that an enhanced N supply decreased aliphatic GSLs. In rape seeds the concentration of S containing amino acids such as methionine, the precursor amino acid for aliphatic GSLs synthesis, increased with an increased S supply, and this response was more pronounced at high N supply resulting in an increasing GSL concentration.[26] Although a negative correlation was found between N concentration and aliphatic GSLs in broccoli florets, the reduction of N concentration in intercropped broccoli did not result in an increase of aliphatic GSLs.

In this study the dominant indole GSL in broccoli florets was neoglucobrassicin. Most studies[9,13] have reported glucobrassicin as the main indole GSL in broccoli. Differences in results could be due to differences in broccoli cultivars tested.[5] Omirou et al.[13] showed that a lack of N suppressed indole GSLs in broccoli florets, but in our study N fertilization did not show any clear effect on indole GSLs. However, the decreased indole GSLs concentrations in broccoli in the intercropping system might be attributed to the lower N concentrations in broccoli florets compared to under the sole cropping system. More N is needed to synthesize indole GSLs than aliphatic GSLs because two atoms of N instead of one are needed for the biosynthesis of indole GSLs.[27]

Increasing the N supply without S fertilization decreased the total GSLs independent of the cropping system suggesting that the N uptake by lettuce was not strong enough to increase the relative S availability for GSL formation in broccoli. High N supply was found to increase protein concentration in seeds of *B. napus*, and when S was limited most of the S was incorporated into proteins. Therefore less S was available for glucosinolate synthesis.[28] In both cropping systems total aliphatic and indole GSLs were positively correlated with the S concentrations in broccoli florets, as the S uptake of intercropped broccoli was not lower compared with sole cropped broccoli. The decrease in the total GSLs at high N supply could be partially explained by the tendency to decrease S concentrations in broccoli when N fertilization rate increased. Similar results were obtained by Schonhof et al.[9] who found that in broccoli florets at high N fertilization the total S concentrations decreased. Moreover, the N:S ratio increased and a negative relationship between GSL concentration and N:S ratio was found, as reported before for the turnip[8] and broccoli.[9]

Enhanced S supply increased GSL concentration in several *Brassica* species.[8,12,13] Both total aliphatic and indole GSLs were found to have a positive relationship with S concentration in broccoli florets. Reduced response of GSLs to S fertilization at low N supply was also observed by Omirou et al. [13] in broccoli florets. Glucosinolates are both S and N containing compounds,[27] and N limitation may have restricted both aliphatic and indole GSLs synthesis.

In the field experiment, intercropping and N fertilization influenced N concentrations in broccoli florets. Yildirim and Guvenc[17] and Guvenc and Yildirim[16] have found a non-significant tendency of lower N concentration when cauliflower and cabbage were intercropped with lettuce in an additive design. In our study the lower N concentrations were not seen as an effect of competition, as the partial LER values of broccoli based on total N and S uptake were up to 11 and 10 times higher, respectively, compared to the partial LER values of lettuce. Although N concentrations in lettuce were higher in intercropping, the total N uptake was low due to the limited growth of the lettuce.

The Pot Experiment. Limited information is available concerning the interactive effects of N and S supply on the glucosinolate concentrations in *B. juncea* leaves. Glucosinolate concentrations in red leaf mustard was reduced when N supply increased at low S supply, whereas the opposite effect was observed at high S supply, which is in agreement with reports for the seeds of Indian mustard[29] and those of our field study in broccoli. In contrast to our field experiment, intercropping increased total, indole, and aliphatic GSL concentrations in red leaf mustard. Differences in total GSLs were mainly caused by changes in the main aliphatic glucosinolate sinigrin. The increase of both aliphatic and indole GSLs concentration in intercropping could be attributed to the higher N concentrations in intercropped red leaf mustard, as positive correlation was found between N and GSL concentrations. It is obvious that there exist a species specific relation of aliphatic glucosinolates and N concentrations as the opposite trend was found between them in broccoli at our field experiment. Glucosinolate concentrations in red leaf mustard were not correlated with the N:S ratio. Gerendás et al.[29] found that sinigrin concentration in Indian mustard seeds does not relate to the N:S ratio when S status is excessive or severely limiting. In our study, S concentration in red leaf mustard was higher than the S concentrations found in Indian mustard's leaves.[29]

Dry weight of individual plants of red leaf mustard increased by intercropping. This was mainly because lettuce was not competitive relative to red leaf mustard, as it was also shown by the partial LER values based on DM. In contrast to the field experiment, the competition between the intercropped species occurred only below ground as the above ground competition was successfully eliminated with the use of the Polystyrene foam board. The partial LER values based on N and S uptake reveal that red leaf mustard was more efficient to take up nutrients than lettuce.

Both N and S concentrations in red leaf mustard were influenced by N and S fertilization, respectively, but no clear interaction between N and S fertilizations was observed. Instead an S supply × cropping system interaction was determined for the N concentration, which indicated that at high S supplies, S availability increased in intercropping which may have led to the increased N accumulation observed in mustard plants. Several studies showed that S fertilization enhanced N uptake by rapeseed mustard[30] and oilseed rape.[12] A combined application of S and N increased the total N accumulation in *B. juncea* shoots compared to N application alone.[30] Similarly, Gerendás et al.[29] found strong interactive effects of S and N supply on N concentration in leaves of Indian mustard.

In conclusion, the present research confirmed that GSL concentrations in *Brassicas* may be increased by altering the N:S ratio through appropriate N and S fertilization, and that it may also be affected by intercropping with non-*Brassica* crops, though the lettuce plants were too weak competitors here to achieve a clear test of of this hypothesis in the field study. If intercropping and fertilization can be used to increase GSL concentrations, it can also be used to increase the health benefits when consuming *Brassica* vegetables. The effect of intercropping on GSL concentrations was clearly shown in the pot experiment when the above ground interactions were eliminated. The results indicate a species specific response to intercropping; therefore further work is required to develop efficient intercropping systems. A main factor will be selection of plant material and intercrop design to develop systems where the non-*Brassica* species can develop better than in the present experiments; otherwise, their effect will remain limited.

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Notes

The authors declare no competing financial interest.

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ABREVIATIONS USED

N: nitrogen; S: sulfur; DM: dry matter; HPLC: high-performance liquid chromatography; CFA: continuous flow analysis; GSLs: glucosinolates; PRO: progoitrin; GNA: gluconapin; SIN: sinigrin; GRA: glucoraphanin; GIB: glucoiberin; GBS: glucobrassicin; NGB: neoglucobrassicin; MGB: 4-methoxy-glucobrassicin; LER: Land equivalent ratio

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