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ARTICLE

Brassicaceae Tissues as Inhibitors of Nitrification in Soil

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Brassicaceae crops often produce an unexplained increase in plant-available soil N possibly related to bioactive compounds produced from glucosinolates present in the tissues. Our objective was to determine if glucosinolate-containing tissues inhibit nitrification, thereby potentially explaining this observation. Ammonium, NO_2^-, and NO_3^- N were measured in soils amended with Brassicaceae (*Isatis tinctoria* L., *Brassica napus* L., *Brassica juncea* L., and *Sinapis alba* L.) tissues containing different glucosinolate types and concentrations or Kentucky bluegrass (*Poa pratensis* L.) residues with equivalent C/N ratios as the Brassicaceae samples. There was greater accumulation of NH_4^+ N in soils amended with tissues containing high glucosinolate concentrations as compared to soils amended with tissues containing no or low glucosinolate concentrations. Nitrite N was detected only in soils amended with Brassicaceae tissues having the highest glucosinolate concentrations. The positive correlation of both NH_4^+ and NO_2^- N accumulation with the glucosinolate concentration indicates the participation of glucosinolate hydrolysis products in nitrification inhibition.

KEYWORDS: Glucosinolates; nitrification inhibition; allelochemicals; nitrogen cycle; isothiocyanates; chemical ecology

INTRODUCTION

Brassica and *Sinapis* species in the Brassicaceae are used as rotational or cover crops in an attempt to manage weeds, inhibit other plant pests, improve soil physical properties, decrease erosion, and promote soil fertility (*1*−*4*). In addition to general benefits, species in this family often produce a positive response on the following crop in excess of that demonstrated for other rotational crops (*3*, *4*). For example, it has been shown that inorganic soil N increases during the fallow period following canola (*Brassica napus*) harvest to a greater extent than following cereals and in some instances even legumes (*5*, *6*). Increases in inorganic soil N could not be explained by total tissue N, tissue amounts, or C/N ratios of the tissues.

Possible reasons for greater maintenance of soil N availability include (1) nitrification inhibition, (2) greater mineralization of organic N within the tissues themselves, soil organic matter, or microbial biomass, and (3) reduced microbial immobilization of N. It is unclear as to which of these mechanisms might be most important under field conditions (*5*, *7*). The observed impact on N availability is potentially related to the fact that all members of the Brassicaceae produce glucosinolates, sulfur-containing organic anions that release a number of biologically active products as a result of enzymatic hydrolysis.

In soil amended with Brassicaceae tissues, the predominant glucosinolate degradation products include isothiocyanates, oxazolidinethiones, and SCN^- (*8*−*10*). Glucosinolate hydrolysis products inhibit microorganisms (*11*), and thus, in a plant−soil environment, these products may alter microbial activity and influence N cycling. A dithiocarbamate fumigant that produces methyl isothiocyanate was shown to inhibit nitrification (*12*). Detailed studies using various expected glucosinolate hydrolysis products likewise demonstrated that isothiocyanates inhibit nitrification (*7*). In addition to isothiocyanates, SCN^- has also been shown to inhibit nitrite-forming bacteria (*13*). Field studies indicate that populations of microorganisms responsible for NH_4^+ oxidation are lower in soils after a canola crop as compared to wheat (*6*), further supporting the possibility of delayed nitrification as a consequence of compounds produced by Brassicaceae tissues.

Nitrification inhibition by plant tissues or plant-derived compounds is not without precedent. A variety of nonglucosinolate-containing plant-derived materials have been shown to inhibit nitrification including but not limited to tissues of *Arbutus unedo*, a Mediterranean woodland species (*14*), various products from the mint *Mentha spicata* (*15*), aqueous extracts from *Cynodon dactylon* L. rhizomes (*16*), and extracts of the perennial legume *Astragalus* (*17*). The most detailed studies have been conducted with a tropical pasture grass *Brachiaria humidicola*, with the investigators identifying potentially responsible compounds and promoting the concept that biological nitrification inhibitors are of potential utility in improving N use efficiency in crops (*18*, *19*).

A more complete understanding of how glucosinolate-containing plants impact soil processes is necessary to maximize the benefits of using Brassicaceae plants in rotations or as cover crops and green manures. Our goal was to determine the influence of glucosinolate-containing plant tissues on nitrogen cycling in soil, with the specific objective of determining whether Brassicaceae tissues alter nitrification in a manner consistent with biological activities of the expected hydrolysis products produced by

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Table 1. Glucosinolate Concentrations in μmol g^{-1} Tissue for Glucosinolate-Containing Plant Tissue Amendments

glucosinolate R group	*B. napus* Asp	*I. tinctoria*	*S. alba*	*B. napus* DE	*B. juncea*
propenyl (allyl)[a]	0.00	0.00	0.00	0.00	48.74
1-methylpropyl[a]	0.00	0.00	0.00	trace	0.00
3-butenyl[a]	0.60	1.49	0.54	1.98	0.51
4-pentenyl[a]	0.96	trace	0.00	11.71	0.00
2-hydroxy-3-butenyl[b]	0.61	3.67	1.89	8.59	0.00
2-hydroxy-3-pentenyl[b]	0.16	0.00	0.00	3.74	0.00
4-methylthiobutyl[a]	0.20	0.00	1.36	0.10	0.00
5-methylthiopentyl[a]	0.18	0.00	0.00	0.14	0.00
benzyl[a]	0.00	0.00	6.05	0.00	0.00
4-hydroxybenzyl[c]	0.00	0.00	9.00	0.00	0.00
phenylethyl[a]	2.19	0.00	0.50	2.01	3.49
indolyl-3-methyl[c]	0.65	10.28	trace	0.37	0.24
1-methoxyindolyl-3-methyl[c]	trace	trace	trace	trace	trace
4-hydroxyindolyl-3-methyl[c]	trace	trace	0.06	0.07	0.19
4-methoxyindolyl-3-methyl[c]	trace	0.00	0.50	0.63	trace
isothiocyanate forming	4.13	1.49	8.45	15.90	52.74
SCN^- forming	0.65	10.28	9.56	0.70	0.43
total GS	5.55	15.44	19.90	29.34	53.17

[a] Isothiocyanate-forming glucosinolate. [b] Oxazolidinethione-forming glucosinolate. [c] SCN^--forming glucosinolate.

glucosinolates in those tissues. We approached this objective by comparing NH_4^+, NO_2^-, and NO_3^- production in soils amended with tissues containing different glucosinolate types and concentrations to the same inorganic N species produced in soils amended with non-glucosinolate-containing tissues having similar C/N ratios as the Brassicaceae samples.

MATERIALS AND METHODS

Soil. Soil was collected, air-dried, crushed to pass a 2 mm sieve, and characterized according to procedures described previously (*20*). We used a Latahco silt loam with the following characteristics: pH 6.0, 1.6 mg of N g^{-1}, 16.9 mg of organic carbon g^{-1}, C/N ratio of 10.56, 185 g of clay kg^{-1}, 70 g of sand kg^{-1}, water content at 0.033 MPa of 28.6%, and water content at 0.05 MPa of 25.8%.

Plant Tissues. Glucosinolate-containing plants were field-grown and harvested while plants were flowering. Plants included *Isatis tinctoria* (Dyer's woad), *Sinapis alba* (yellow mustard), *Brassica juncea* (Oriental mustard), and two varieties of *B. napus* (rapeseed), including both high (Dwarf Essex) and low (Aspen) glucosinolate varieties. Tissues were frozen, lyophilized, and ground to pass a 2 mm sieve. Except in the case of *I. tinctoria* for which there were only tops, both tops and roots were included to make a whole-plant amendment. Glucosinolates were extracted, identified using coupled gas chromatography (GC)−mass spectrometry, and quantified using GC in combination with a flame ionization detector (FID) (*21*). FID response factors were assumed to correspond to carbon number. GC-operating conditions were modified to include: injector, 270 °C; detector, 305 °C; initial oven temperature, 130 °C for 1 min, ramped 18 °C/min to 220 °C, 8 °C/min to 300 °C, and held for 10 min; purge (splitless injection), 0.5 min; and He head pressure, 60 kPa. The column coating was 5% phenyl-substituted methylpolysiloxane (DB-5, J&W Folsom, CA; 30 m, 0.25 mm inner diameter, 0.250 μm film). Glucosinolate concentrations in the plant materials are shown in **Table 1**.

Kentucky bluegrass (*Poa pratensis* L.) grown with various applications of N fertilization was used to obtain nonglucosinolate-containing plant tissues having similar C/N ratios as the glucosinolate-containing material. These plant tissues served as another type of control to account for added N from plant material and the effects of the C/N ratio on nitrification exclusive of glucosinolate hydrolysis products.

Total C and N analysis was performed on all plant tissues using a combustion technique (CHN analyzer, Leco Corporation, St. Joseph, MI). Plant tissue C and N characteristics are shown in **Table 2**.

Soil Amendment and Incubation. Three separate incubation experiments were performed. In one set of experiments, soils were incubated

Table 2. Carbon and Nitrogen Contents of Plant Tissues Used as Soil Amendments

soil and tissue amendment	total N (mg g^{-1} tissue)	organic carbon (mg g^{-1} tissue)	C/N ratio
I. tinctoria	23.5	410	17.45
P. pratensis 1[a]	25.2	427	16.94
P. pratensis (4 day)[b]	25.5	400	15.69
B. napus Aspen	29.3	400	13.65
P. pratensis 2	30.4	399	13.11
B. napus Dwarf Essex	36.5	380	10.41
B. juncea	35.9	370	10.31
S. alba	38.7	350	9.04
P. pratensis 3	47.3	427	9.03

[a] Numerical designations following the species name indicate plants grown with different amounts of N fertilization to obtain nonglucosinolate-containing tissues with a range in C/N ratios. [b] Length of the experimental incubation.

4 days, whereas in the remaining two incubations, soils were incubated 24 days. The shorter 4 day incubation was included specifically to address the possibility and transitory nature of NO_2^- formation. Urea (carbonyl diamide) fertilizer was added in the 4 day and one of the 24 day incubations as an extra nitrogen source that could be readily mineralized and undergo subsequent nitrification in the soil environment. This form of N was chosen given its common use as a N fertilizer (*22*). In the 4 day incubation, soil was amended with 40 μg of urea N g^{-1} of soil, and in one of the 24 day incubations, soil was amended with 70 μg of urea N g^{-1} of soil. The other 24 day treatment had no urea addition. Each 40 g air-dried soil sample, except for the control (unamended) and control plus urea, was amended with 0.4 g of plant tissue (**Table 1**) (1% tissue/soil, dry wt/wt), providing from 235 μg of N g^{-1} of soil for *I. tinctoria* to 473 μg of N g^{-1} of soil for *P. pratensis* 3 (**Table 2**). Deionized water was added to non-tissue-amended soils in an amount of 10.3 mL (25.75%) to produce a soil−water potential of 0.05 MPa. Increased water in the amount of 11.5 mL was added to tissue-amended soils (28.5%) to compensate for added plant tissue and, thereby, also produce a water potential of 0.05 MPa. The moisture content during the incubations was maintained by adding water to a constant weight on a daily basis. Moisture contents measured at 24 days ranged between 23 and 28%. Soil samples were incubated between 18 and 20 °C in uncapped glass bottles. Duplicate samples were included in the 4 day experiment, and triplicate samples were included in the 24 day experiments.

Soil Nitrogen Analysis. Samples of 3.5−4.5 g of moist soil were obtained by collecting a complete top to bottom core through the depth of the soil using a 1.6 cm diameter metal tube. Samples were then extracted with 30 mL of 2.0 M KCl by shaking for 1 h followed by centrifugation for 10 min (670*g*) and filtration (Whatman No. 42) of the supernatant. Nitrite, NO_3^-, and NH_4^+ N were quantified in the 4 day incubation experiment using automated QuickChem colorimetric methods 12-107-06-2-A and 12-107-04-1-B (*23*). In the 24 day experiment, only NO_2^- N was determined in extracts using the automated procedure. Nitrate and NH_4^+ N were instead determined by modified Kjeldahl methods (*24*). Urea was quantified using a colorimetric procedure (*25*), but because it was essentially hydrolyzed in 4 days, urea was not analyzed at 24 days.

Statistical Analysis. Data were analyzed with one-way analysis of variance (ANOVA) using SYSTAT software. The one factor was soil amendment. Each ANOVA was followed by a least significant difference (LSD) multiple comparisons test to determine specific differences between soil treatments. A multiple comparisons test was performed only if the ANOVA was significant.

RESULTS

Urea hydrolysis in the 4 day experiment was nearly complete, with all treatments showing the presence of less than 10% ($\leq$3.7 μg of urea N g^{-1} of soil) of the original amount of urea added (40 μg of urea N g^{-1} of soil) (**Figure 1**). Greater NH_4^+ N accumulated in plant-tissue-amended soils as compared to the unamended soil and the urea-amended soil, except in the case of *I. tinctoria*. Nitrite accumulation 4 days after the addition of urea occurred exclusively in those soils amended with tissues

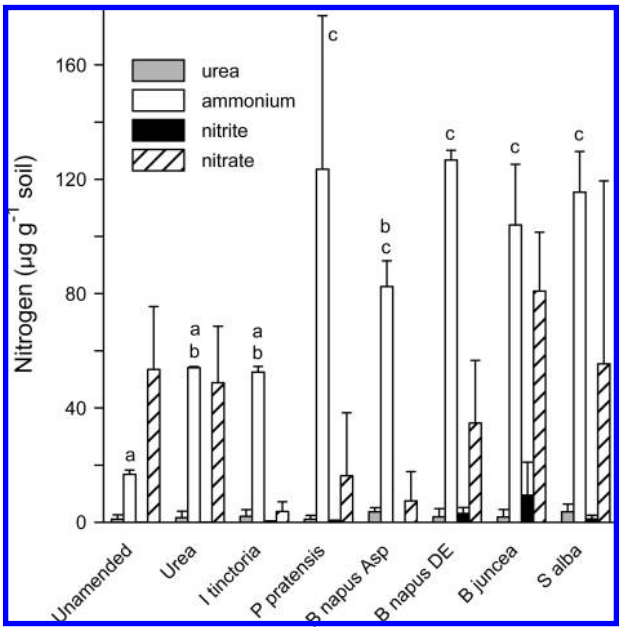

Figure 1. Amount of each N-containing species extracted from Latahco silt loam soil 4 days after amendment with the noted tissue and urea (40 μg g^{-1} soil). Error bars represent + 1 standard deviation. Plant-tissue-amended treatments are shown in order of decreasing C/N ratios. Except for ammonium, multiple comparison tests were not performed because of a lack of significant ANOVAs ($p \leq 0.05$). *P. pratensis* tissue used in this experiment is designated in **Table 2** by the 4 day incubation time.

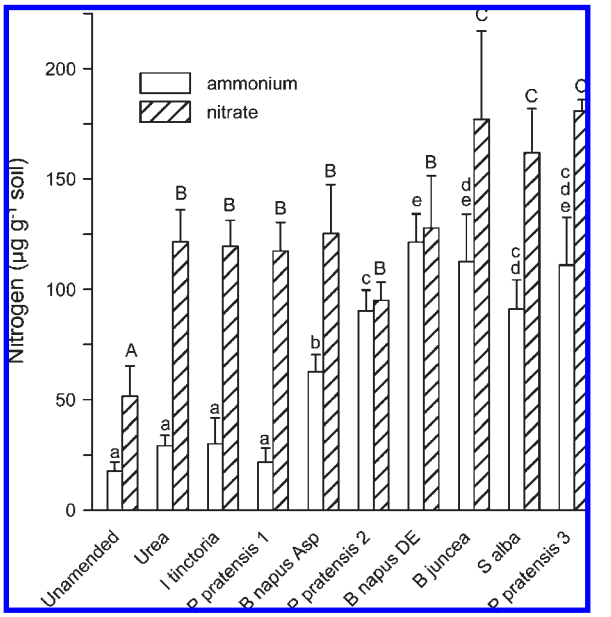

Figure 2. Amount of each N-containing species extracted from Latahco silt loam soil 24 days after amendment with the noted tissue and urea (70 μg g^{-1} soil). Error bars represent + 1 standard deviation. Plant-tissue-amended treatments are shown in order of decreasing C/N ratios. Mean concentrations of a single species designated with the same letter are equivalent at $p \leq 0.05$. Numerical designations following *P. pratensis* indicate plants grown with different amounts of N fertilization.

containing the highest glucosinolate concentrations (*B. juncea*, *B. napus* Dwarf Essex, and *S. alba*) (**Figure 1**). The greatest amount of NO_2^- N accumulated in soil amended with *B. juncea* tissues containing high concentrations (48.74 μmol g^{-1} tissue) of a glucosinolate that releases 2-propenyl isothiocyanate (**Table 1**). This tissue contained 3−6 times the concentration of isothiocyanate-producing glucosinolates (52.74 μmol g^{-1} tissue) as the two remaining tissues that caused NO_2^- N accumulation. Neither *B. napus* nor *S. alba* tissues contain glucosinolates that are expected to produce 2-propenyl isothiocyanate (**Table 1**). Amendments not producing a NO_2^- N accumulation (*I. tinctoria* and *B. napus* Aspen) contained less than one-tenth the concentrations (< 5 μmol g^{-1} tissue) of isothiocyanate-producing glucosinolates as *B. juncea*. There was a nonstatistically significant trend for less NO_3^- N production in soils amended with *I. tinctoria*, *P. pratensis*, and *B. napus* Aspen as compared to the nontissue-amended treatment (**Figure 1**), because of high C/N ratios in these tissues (**Table 2**).

At 24 days, the unamended soil remained approximately the same in its N distribution profile as the 4 day treatment but, in the urea-amended soils, much of the NH_4^+ N had been converted to NO_3^- N (**Figure 2**). Neither urea nor NO_2^- N was detected in any of the tissue-amended soils to which urea was added (**Figure 2**). More NH_4^+ N was extracted from soils amended with tissues having C/N ratios equal to or less than 13.11 (*P. pratensis* 2, *B. napus* Dwarf Essex, *B. juncea*, *S. alba*, and *P. pratensis* 3) as compared to those soils amended with tissues having C/N ratios of 13.65 and greater (*I. tinctoria*, *P. pratensis* 1, and *B. napus* Aspen) (**Figure 2** and **Table 2**). Similarly, except for *B. napus* Dwarf Essex, more NO_3^- N was extracted from soils amended with tissues having lower C/N ratios (*B. juncea*, *S. alba*, and *P. pratensis* 3). The one-way ANOVAs for NO_3^- N and NH_4^+ N were both significant at $p < 0.001$. Differences in extractable N among soils amended with tissues containing high glucosinolate concentrations compared to those amended with

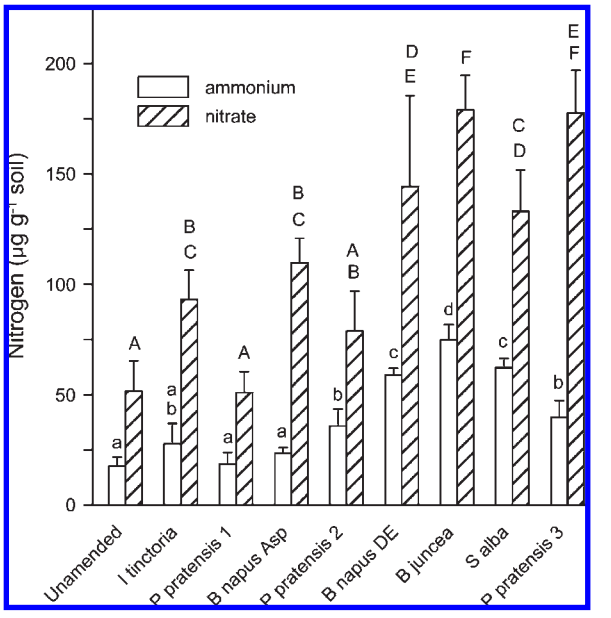

Figure 3. Amount of each N-containing species extracted from Latahco silt loam soil 24 days after amendment with the noted tissue. Error bars represent +1 standard deviation. Plant-tissue-amended treatments are shown in order of decreasing C/N ratios. Mean concentrations of a single species designated with the same letter are equivalent at $p \leq 0.05$.

P. pratensis samples having comparable C/N ratios were not observed.

In the 24 day incubation without urea amendment, NO_2^- N was absent, consistent with the 24 day experiment, in which urea was included (**Figure 3**). Amounts of NH_4^+ N extracted from soils amended with plants containing low glucosinolate concentrations (*I. tinctoria* and *B. napus* Aspen) were equal or less than those

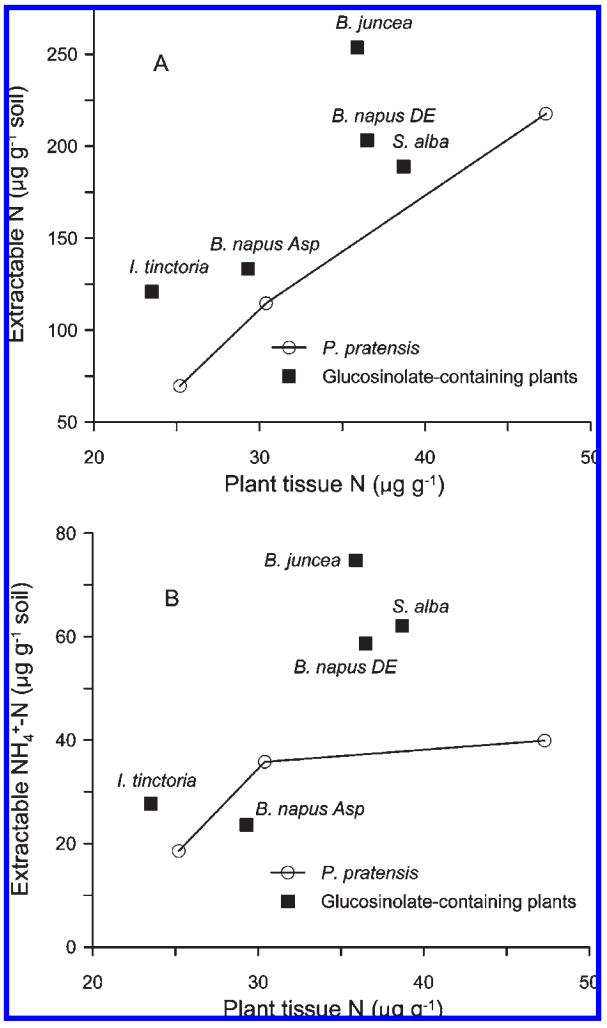

Figure 4. (A) Total N and (B) NH_4^+ N extracted from Latahco silt loam soil 24 days after amendment with the noted tissue plotted as a function of plant tissue N. Lines connect the three *P. pratensis* samples that vary in N content.

from soils amended with *P. pratensis* 1 or 2 having a similar C/N ratio (**Figure 3** and **Table 2**). This was not the case for soils amended with tissues containing higher glucosinolate concentrations. More NH_4^+ N was extracted from soils amended with *B. napus* Dwarf Essex, *B. juncea*, and *S. alba* as compared to soils amended with *P. pratensis* of similar C/N ratios. Extractable NO_3^- N concentrations of soils amended with *B. napus* Dwarf Essex and *B. juncea* were similar to those extracted from *P. pratensis*-3-amended soils and slightly less for *S. alba*.

The trend in the 24 day incubation without urea for more extractable N (NO_2^- + NO_3^- + NH_4^+ N) from high glucosinolate-containing amendments as compared to the *P. pratensis* cannot be explained by greater plant tissue N concentrations (**Figure 4A**). Instead, this difference is largely a function of greater extractable NH_4^+ N (**Figures 3** and **4B**). Soils amended with *B. juncea* tissues produced twice as much extractable NH_4^+ N as those amended with *P. pratensis* tissues of comparable C/N ratios (**Figure 3**) as well as total tissue N concentrations (**Figure 4B**). Likewise, about 50% more NH_4^+ N was extracted from soils amended with *B. napus* Dwarf Essex or *S. alba* tissues than *P. pratensis*-amended soils. Thus, the consistent trend for the extraction of more NH_4^+ N from soils amended with tissues containing relatively high glucosinolate concentrations was not a function of greater total N in those tissues or of lower C/N ratios than the *P. pratensis* tissues to which they were compared. These results contrast with those obtained for urea-amended soils incubated for 24 days (**Figure 2**), because the much larger amounts of NH_4^+ N formed as a result of urea hydrolysis masked any detectable differences.

DISCUSSION

Results presented here show that nitrification inhibition, as indicated by accumulations of NH_4^+ and NO_2^- N, positively correlated with glucosinolate concentrations of the amended plant tissues. The observed increases in extractable concentrations of NH_4^+ and NO_2^- N occurred with tissues of three different Brassicaceae species, each of which contained different types of glucosinolates (**Table 1**). This implies that multiple glucosinolate hydrolysis products are capable of inhibiting nitrification.

Plant tissues producing the greatest delay in nitrification (*B. juncea*, *B. napus* DE, and *S. alba*) contained the highest concentrations of isothiocyanate-forming glucosinolates (**Table 1**). Isothiocyanates interact with sulfhydryl groups of proteins (*26*, *27*), thereby behaving as general biocides. Isothiocyanates are known to inhibit microbial activity and have been implicated in both the inhibition of nitrification and nitrifying organisms (*7*, *12*). Low glucosinolate *B. napus* Aspen was the only amendment to result in less extractable NH_4^+ N than *P. pratensis* amendments of comparable N content (**Figure 4B**), consistent with low isothiocyanate production and little inhibition of nitrification. The pulse of NO_2^- N at 4 days was also associated only with those tissues containing greater concentrations of isothiocyanate-producing glucosinolates, suggesting that bacteria responsible for NH_4^+ oxidation to NO_2^- may be less susceptible to inhibition than those organisms oxidizing NO_2^- to NO_3^-. The absence of NO_2^- N in longer incubations is consistent with the findings that the addition of an isothiocyanate-forming dithiocarbamate to soil inhibited nitrification at 16 days but not at 30, 44, 58, and 76 days (*12*).

There was a trend for greater accumulations of both NH_4^+ N and NO_2^- N in soil amended with *B. juncea* tissues producing 2-propenyl isothiocyanate as compared to soil amended with *B. napus* Dwarf Essex or *S. alba* tissues (**Figures 3** and **4B**). This distinction is potentially a function not only of the greater amount of isothiocyanate formed by *B. juncea* tissues but also the differential behavior, biological availabilities, and toxicities of the hydrolytic products from the different tissues. *B. juncea* tissues contain primarily 2-propenyl glucosinolate, whereas *S. alba* tissues contain benzyl and *B. napus* Dwarf Essex contain 4-pentenyl as the predominanat isothiocyanate-forming glucosinolates (**Table 1**). Each of the respective isothiocyanates produced from these glucosinolates has its own vapor pressure and hydrophobicity that govern not only movement and sorption within the three-phase soil environment but also transport through cellular membranes and toxicity to the target organism (*28*). Bending and Lincoln (*7*) showed differences in nitrification inhibition among six different isothiocyanates, but there was no consistent trend with respect to compound aromaticity. In contrast, isothiocyanate polarity positively correlated with toxicity to black vine weevil [*Otiorhynchus sulcatus* (F.)], a soil-borne insect pest (*28*). It therefore should not be assumed that direct comparisons among absolute concentrations of isothiocyanate-forming glucosinolates and observed effects on nitrification are possible. Some isothiocyanates may more effectively inhibit nitrification than others, depending upon both bioavailability in soil and inherent toxicity of the molecule itself.

In addition to isothiocyanates, glucosinolates produce other hydrolysis products that may impact N cycling. SCN^- as

produced during the hydrolysis of both indolylic and 4-OH benzyl glucosinolates (*29*) and oxazolidinethiones from aliphatic glucosinolates with a β-hydroxyl group are generally less biologically active than isothiocyanates (*11*). SCN^- was shown to inhibit nitrite-forming bacteria, but the concentrations necessary to achieve such inhibition are greater than those expected from Brassicaceae green manures (*13*). SCN^- is the only major glucosinolate hydrolysis product expected from *I. tinctoria*, whereas both SCN^- and isothiocyanates are expected from *S. alba* tissues (**Table 1**). The lack of NO_2^- N accumulation in soils amended with *I. tinctoria* and the presence of NO_2^- N in *S. alba*-amended soils correlates with this distinction in glucosinolates.

In addition, glucosinolate products, such as isothiocyanates, oxazolidinethiones, and SCN^-, may further degrade, producing still other S-containing biologically active compounds. For example, one of the main routes for SCN^- biodegradation is the carbonyl pathway, in which carbonyl sulfide (COS) is produced (*30*). There are several reports of COS emissions from soils amended with SCN^- salts (*31*−*33*), substantiating the likelihood that the carbonyl pathway is of importance in the environment. Alternatively, recent investigations on coke- and steel-processing wastewaters indicate that SCN^- itself is not directly inhibitory to nitrifying bacteria but that toxicity stems from NH_3 produced as a result of SCN^- degradation (*34*). Although our knowledge of the specific pathways is limited, additional S-containing compounds linked to sulfur metabolism in soil, such as thiosulfate and CS_2, also inhibit nitrification (*35*−*37*). Bending and Lincoln (*38*) detected CS_2 formation by glucosinolate-containing tissues but concluded it originated from S-containing amino acids instead of isothiocyanates. This suggests the possibility that additive and synergistic effects may result through the simultaneous production of both glucosinolate hydrolysis products and additional compounds unrelated to glucosinolates. Synergistic effects of combining 2-propeny isothiocyanate and dimethyl sulfide, another potential product from plant tissues, on nitrification inhibition have been reported (*7*).

Although direct inhibition of nitrifying organisms is most likely, isothiocyanates are general biocides that potentially inhibit heterotrophic microorganisms as well (*11*). As such, NH_4^+ N accumulation in soils amended with glucosinolate-containing tissues might also result from both greater N release from any killed biomass and, correspondingly, reduced immobilization by the remaining biomass. The observed increase in NH_4^+ N could therefore result from the combined action of these two mechanisms along with nitrification inhibition. However, the lack of an associated increase in NO_3^- N and the detection of NO_2^- N suggest that nitrification inhibition was the dominant process.

Our research indicates that those Brassicaceae crops containing the highest isothiocyanate-producing glucosinolate concentrations are most likely to inhibit nitrification and increase plant-available N. Efforts to increase plant-available soil N using Brassicaceae species will thus most likely benefit to the greatest extent by including varieties with the highest concentrations of glucosinolates expected to produce isothiocyanates upon their hydrolysis. The concept of using biological nitrification inhibitors derived from non-glucosinolate-containing plants to improve N use efficiency in crops has been proposed (*18*).

The length of time that nitrification inhibition is realized in a field situation is unknown; however, root exudation of glucosinolates (*39*) or their degradation products (*40*) is another possible route of influence that might extend such effects. It was recently shown that a metal-accumulating *Thlaspi* species of Brassicaceae produced rhizosphere soil that had several higher measures of biological activity than non-rhizosphere soil but lower nitrification potential (*41*). However, it is important to remember that glucosinolate hydrolysis products, such as isothiocyanates, act as general biocides. Thus, although we have demonstrated the potential for Brassicaceae tissue to delay nitrification, dramatic single effects may not explain overall ecological impacts that result from complicated synergistic or additive processes, as occur with tissue incorporation into soil.

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