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Distribution of Glucosinolates in *Brassica oleracea* Cultivars

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Summary

Castro A., Aires A., Rosa E., Bloem E., Stulen I. & De Kok L. J. 2004. Distribution of glucosinolates in *Brassica oleracea* cultivars. – Phyton 44 (1): 133–143, with 3 figures. English with German summary.

Glucosinolates are important secondary compounds, commonly found in *Brassicaceae*. To what extent these sulfur and nitrogen containing compounds contribute to the total sulfur pool was investigated. In various cultivars of *Brassica oleracea* viz. curly kale, pointed cabbage, red cabbage, savoy cabbage and white cabbage, total glucosinolate content and composition of the glucosinolate pool (aliphatic, aromatic and indolyl glucosinolates) were measured, together with determination of the total and inorganic sulfur and nitrogen contents. Differences in the contents of the individual glucosinolates were found between cultivars and plant parts. In all cultivars the total glucosinolate content was highest in the roots. Roots of curly kale had the highest gluconasturtiin content (12.6 μmol g^{-1} DW), while the shoot of white cabbage had the lowest glucoiberin content (0.3 μmol g^{-1} DW). Between all cultivars significant differences in glucosinolate content per class were found. Aromatic glu-

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cosinolates were highest in the roots of curly kale, accounting for 63% of the total content, while the roots of savoy cabbage had the highest aliphatic glucosinolate content (8.1 μmol g^{-1} DW). Expression of the total glucosinolate fraction on a total sulfur basis showed that the contribution of the glucosinolate fraction was very low (1–2%). Since a large amount of total sulfur was present as sulfate (70–88%) total glucosinolate content was also expressed on an organic sulfur basis. These values ranged between 10–23%, for white cabbage and savoy cabbage, respectively. We conclude that expression on an organic sulfur basis is a better parameter to assess the fraction of glucosinolates than on a total sulfur basis. In addition, the organic fraction appears to be a better parameter to define the sulfur requirement for structural growth.

Zusammenfassung

Castro A., Aires A., Rosa E., Bloem E., Stulen I. & De Kok L. J. 2004. Glucosinolatverteilung in unterschiedlichen Kulturformen von *Brassica oleracea*. – Phyton 44 (1): 133-143, 3 Abbildungen. Englisch mit deutscher Zusammenfassung.

Glucosinolate sind wichtige sekundäre Pflanzeninhaltsstoffe, die sich für gewöhnlich bei allen Brassicaceen finden. In der vorliegenden Studie wurde untersucht, wie hoch der Anteil dieser schwefel- und stickstoffhaltigen Inhaltsstoffe am Gesamtschwefelpool der Pflanzen ist. In verschiedenen Kulturformen von *Brassica oleracea* und zwar Grünkohl, Rotkohl, Weißkohl, Wirsingkohl und Spitzkohl wurde dazu der Gesamtglucosinolatgehalt sowie die Zusammensetzung der Einzelglucosinolate (aliphatische, aromatische, und Indolglucosinolate) bestimmt sowie der Gesamtschwefel- und Stickstoffgehalt und der Anteil an anorganischem Schwefel in den Pflanzen.

Unterschiede in der Zusammensetzung der Einzelglucosinolate wurden sowohl zwischen den verschiedenen Kulturformen ermittelt als auch in unterschiedlichen Pflanzenteilen. Der höchste Glucosinolatgehalt fand sich bei allen Cultivaren in den Wurzeln. Die Wurzeln vom Grünkohl hatten den höchsten Gehalt an Gluconasturtiin (12.6 μmol g^{-1} DW), während der Spross vom Weißkohl den niedrigsten Glucoiberingehalt (0.3 μmol g^{-1} DW) aufwies. Zwischen allen Cultivaren fanden sich signifikante Unterschiede in der Verteilung der unterschiedlichen Glucosinolatgruppen. Der höchste Anteil an aromatischen Glucosinolaten mit 63% fand sich in der Wurzel von Grünkohl, während bei Wirsingkohl die aliphatischen Glucosinolate (8.1 μmol g^{-1} DW) in den Wurzeln dominierten.

Bezogen auf den Gesamtschwefelgehalt in den Pflanzen war der Anteil an Schwefel, der in den Glucosinolaten gebunden war, mit 1–2% nur sehr gering. Der Hauptanteil des Schwefels (70–88%) lag in Form von Sulfat in der Pflanze vor.

Bezogen auf den organischen Schwefel in der Pflanze machten die Glucosinolate 10–23% aus. Es erscheint daher sinnvoller, die Fraktion der Glucosinolate zum organischen Schwefel in Bezug zu setzen und nicht zum Gesamtschwefel. Darüber hinaus ist die organische Fraktion besser geeignet, den Schwefelbedarf für das Strukturwachstum der Pflanzen zu definieren.

Introduction

Glucosinolates are secondary sulfur and nitrogen containing metabolites commonly found in *Brassicaceae*, which include economically im-

portant crops as cabbage (*Brassica oleracea*) and oilseed rape (*Brassica napus*) (SCHNUG 1990, 1993, ROSA 1997, GRASER & al. 2001, PETERSEN & al. 2002, REICHELT & al. 2002, WITTSTOCK & HALKIER 2002). There is a great diversity in glucosinolates because of differences in amino acid derived side chains and their elongated derivatives. The synthesis of glucosinolates comprises the oxidation of the parent amino acid to an aldoxime, followed by the addition of a thiol group, most likely as the result of conjugation with cysteine to produce thiohydroximate. Furthermore, the transfer of glucose and sulfate moieties completes the formation of the glucosinolates, which is composed of a β-thioglucose moiety, a sulfonated oxime and a side chain (SCHNUG 1990, ROSA 1997, 1999, GRASER & al. 2001).

The physiological significance of glucosinolates is still obscure, though these secondary sulfur compounds are considered to act as sink compounds in situations of sulfur excess (SCHNUG 1990, 1993, ERNST 1993). However, when *Brassica* was exposed to H_2S (WESTERMAN & al. 2001) and *Arabidopsis* to SO_2 (VAN DER KOOIJ & al. 1997), the sink capacity of the glucosinolate fraction seemed to be rather limited. Upon tissue disruption, glucosinolates are degraded by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to yield glucose, sulfate and aglucone. By fragmentation and/or molecular rearrangements a variety of biologically active products such as isothiocyanates, thiocyanates, nitriles and oxazolidine-2-thiones are formed (ROSA 1997, 1999, KUSHAD & al. 1999, GRASER & al. 2001, PETERSEN & al. 2002, REICHELT & al. 2002, WITTSTOCK & HALKIER 2002). The glucosinolate-myrosinase system is assumed to play a role in plant-herbivore and plant-pathogen interactions. Furthermore, glucosinolates are responsible for the flavour properties of *Brassicaceae* and recently have received attention in view of their potential anticarcinogenic properties (KUSHAD & al. 1999, GRASER & al. 2001, PETERSEN & al. 2002, REICHELT & al. 2002).

In general species of the *Brassicacea* are considered to have a high sulfur requirement for growth. However, in seedlings a large proportion of total sulfur may be present as sulfate. Therefore the definition of the actual sulfur requirement for structural growth may have to be redefined (CASTRO & al. 2003). Little is known about the proportion of sulfur present in glucosinolates in the seedling stage. The present study presents the total glucosinolate content and composition (aliphatic, aromatic and indolyl glucosinolates) in various cultivars of *Brassica oleracea* viz. curly kale, pointed cabbage, red cabbage, savoy cabbage, and white cabbage. The glucosinolate content is expressed as fraction of the organic and total sulfur content.

Material and Methods

Seeds of 5 cultivars of *Brassica oleracea* L. (curly kale, cv. Arsis, Royal Sluis, The Netherlands; pointed cabbage, cv. Duchy F1, Nickerson-Zwaan, The Nether-

lands; red cabbage cv. Rodon F1, Nickerson-Zwaan, The Netherlands; savoy cabbage, cv. Tarvoy F1, Nickerson-Zwaan, The Netherlands; white cabbage, cv. Castello F1, Nickerson-Zwaan, The Netherlands) were germinated in vermiculite in a climate controlled room. Day and night temperatures were 20 and 16 °C respectively, with a relative humidity of 60–70%. The photoperiod was 14 h, at a photon flux rate of 250–300 μmol m^{-2} s^{-1} (photon fluence rate within the 400–700 nm range), supplied by Philips HPI-T (400 W). Twelve-day old seedlings were transferred to 30 l containers (60 plants per container) with 25 % Hoagland nutrient solution (1.25 mM $Ca(NO_3)_2.4H_2O$, 1.25 mM KNO_3, 0.25 mM KH_2PO_4,0.5 mM $MgSO_4.7H_2O$, 11.6 μM H_3BO_3, 2.3 μM $MnCl_2.4H_2O$, 0.19 μM $ZnSO_4.7H_2O$, 0.08 μM $CuSO_4.5H_2O$, 0.13 μM $Na_2MoO_4.2H_2O$, 22 μM Fe(EDTA)), pH 6.

Plants were harvested 4 h after the start of the photoperiod, at day 12, 19 and 26 after sowing (n = 9). At each harvest fresh weight of shoots and roots was determined. Dry weight was determined after freeze-drying. Relative growth rate (RGR) of the total plant was calculated from the ln-transformed fresh weight data of the whole plant between day 19 and 26 (HUNT 1982). Shoot to root (S/R) ratio was calculated on a dry weight basis. The freeze-dried material was used for the chemical analyses. Total nitrogen content was measured in both shoots and roots, using the Kjeldahl method according to BARNEIX & al. 1988. Total sulfur content was measured using a modification of the method from JONES 1995 as described by DURENKAMP & DE KOK 2002. Sulfate and nitrate were extracted according to STUIVER & al. 1992, by refractometric determination after HPLC separation (DURENKAMP & DE KOK 2002). The organic sulfur and nitrogen fractions were calculated by subtracting the sulfate and nitrate fraction from the total sulfur and nitrogen fractions, respectively. Glucosinolates analyses were performed in 26-day-old-seedlings according to PEREIRA & al. 2002. Benzyl glucosinolate was kindly offered by Dr. R. IORI (Instituto Sperimentale Colture Industriali, Via di Corticella, 133, 40129 Bologna, Italy); other glucosinolate standards used for HPLC were isolated and identified according to published methods (PRESTERA & al. 1996, FAHEY & al. 1997, TROYER & al. 2001).

Data for growth parameters, nitrogen and sulfur pools and glucosinolate content was statistically tested by using an unpaired Student's t-test.

Results and Discussion

Growth parameters, sulfur and nitrogen content

Although small differences in relative growth rate (RGR) between the 5 cultivars of *Brassica oleracea* L. were found, they were not significant. The highest shoot to root (S/R) ratios were found in pointed cabbage and savoy cabbage. Savoy cabbage had the highest dry matter content (DMC) and white cabbage the lowest (Table 1). Pointed cabbage and savoy cabbage had the lowest total sulfur content. In all cultivars the main fraction of total sulfur consisted of sulfate. The sulfate fraction accounted for up to 94% in white cabbage, and 90% in pointed cabbage (Fig. 1A). The fact that *Brassicacea* species originate from saline and sulfur enriched environments (WESTERMAN & al. 2001), might explain the high sulfate levels, since these plant species are able to store high quantities of sulfate in the va-

cuole. Savoy cabbage had the highest total nitrogen content, while no differences were found between the other cultivars (Fig. 1B). In savoy cabbage the inorganic nitrogen fraction (nitrate) was 36% of the total nitrogen fraction. In curly kale, which had a lower total nitrogen content, the nitrate fraction was in the same range (32%). The total N / total S ratio ranged from 7.3 to 9.9 for pointed cabbage and savoy cabbage, respectively. No significant differences were found.

Sulfur and nitrogen requirement for growth can be calculated from data on RGR and total sulfur and nitrogen content. Because of the extremely high content of inorganic sulfur found in these *Brassica* cultivars, also reported by DE KOK & al. 2000 and WESTERMAN & al. 2001, the concept of "requirement for growth" may have to be re-defined. For "structural growth" the organic sulfur and nitrogen contents might be better parameters.

Table 1. Growth parameters in different cultivars of *Brassica oleracea*. Data represent the mean of 3 measurements, with 3 plants in each (± SD). RGR, relative growth rate (% day^{-1}) was determined over a two-week interval and is expressed on a plant basis (% fresh weigh day^{-1}); S/R ratio, shoot to root ratio on a dry weight basis; DMC, dry matter content of the plant (%). Means followed by the same letter are not statistically different at $P < 0.01$.

	RGR	S/R ratio	DMC
Curly kale	24.4	6.6 ± 0.3^{a}	9.1 ± 0.4^{b}
Pointed cabbage	22.4	8.0 ± 0.2^{b}	8.4 ± 0.6^{b}
Red cabbage	23.8	6.9 ± 0.3^{a}	8.3 ± 0.6^{cb}
Savoy cabbage	23.4	7.7 ± 0.4^{b}	10.3 ± 0.2^{c}

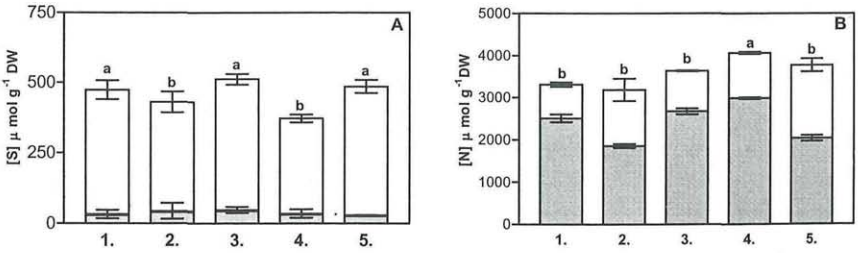

Fig. 1. Sulfur (A) and nitrogen content (B) of *Brassica oleracea* cultivars. The organic fraction is represented in grey (■) and the inorganic fraction in white bars (□). 1. Curly kale, 2. Pointed cabbage, 3. Red cabbage, 4. Savoy cabbage, 5. White cabbage. Data represent the mean of 3 measurements, with 3 plants in each (± SD) and are expressed on a plant dry weight basis. Data on total content followed by the same letter are not statistically different at $P < 0.01$.

Individual glucosinolate content, amino acids composition and glucosinolate content per class

The differences observed in the glucosinolate content between the five *Brassica* cultivars, individual and per class, were apparently due to cultivar genotype, since the cultivars were grown at the same environmental conditions and harvested at the same ontogenic stage (ROSA 1997, KUSHAD & al. 1999, PEREIRA & al. 2002).

The biosynthesis of glucosinolates occurs in different organs of the plant (SCHNUG 1990, 1993, ROSA 1997, KUSHAD & al. 1999, PEREIRA & al. 2002). In the present experiments significant differences in contents of individual glucosinolates between shoot and roots as well as between cultivars were found (Table 2). For curly kale, gluconasturtiin was detected in the highest amounts in the roots, whereas in the shoot progoitrin, 4-hydroxyglucobrassicin and gluconasturtiin were not detected. In red cabbage, progoitrin content in the roots was the highest detected while in the shoot 4-hydroxyglucobrassicin and gluconasturtiin were not detected. Pointed cabbage had the highest gluconasturtiin content in the root, while in the shoot progoitrin and 4-hydroxyglucobrassicin were not detected. In white cabbage, sinigrin was the glucosinolate present at the highest amount in the roots, while in the shoot the same glucosinolates as in curly kale were not detected. Glucosinolate analysis in savoy cabbage showed that sinigrin was the main compound present in the roots while only pro-

Table 2. Content of individual glucosinolates in 5 cultivars of *Brassica oleracea*; curly kale cv. Arsis; red cabbage cv. Rodon; pointed cabbage cv. Duchy; white cabbage, cv. Castello; savoy cabbage cv. Tarvoy. Data is expressed in μmol g^{-1} DW and represents the mean of 3 measurements with 3 plants in each (± SD). S, shoot; R, roots; n.d., not detected; 1, Glucoiberin; 2, Progoitrin; 3, Sinigrin; 4, 4-Hydroxyglucobrassicin; 5, Glucobrassicin; 6, Gluconasturtiin. 1–3 Aliphatic glucosinolates; 4–5 Indolyl glucosinolates; 6. Aromatic glucosinolate. Total glucosinolate content was calculated by adding the content of individual glucosinolate, for each cultivar. Means followed by the same letter are not statistically different at $P < 0.01$.

		1	2	3	4	5	6	Totals
Curly kale	S	1.3±0.0^{a}	n.d.	1.1±0.0^{a}	n.d.	1.1±0.1^{a}	n.d.	3.5^{a}
	R	0.7±0.2^{a}	0.3±0.0^{a}	2.3±0.6^{a}	0.5±0.3^{a}	3.7±0.2^{a}	12.6±0.9^{a}	20.1^{a}
Pointed cabbage	S	1.4±0.0^{a}	n.d.	0.8±0.0^{b}	n.d.	0.5±0.0^{a}	0.2±0.0^{a}	17.3^{b}
	R	0.6±0.0^{b}	1.4±0.0^{b}	1.8±0.0^{b}	0.6±0.0^{b}	1.6±0.0^{b}	5.6±0.3^{b}	11.6^{b}
Red cabbage	S	0.7±0.0^{b}	0.9±0.0^{e}	2.1±0.0^{c}	n.d.	0.9±0.1^{a}	n.d.	4.6^{c}
	R	n.d.	2.4±0.0^{c}	1.1±0.0^{c}	0.1±0.0^{c}	0.2±0.0^{c}	1.0±0.0^{b}	4.8^{c}
Savoy cabbage	S	1.4±0.1^{a}	n.d.	1.5±0.1^{d}	0.1±0.0^{c}	3.7±0.3^{a}	0.2±0.0^{a}	6.9^{d}
	R	1.1±0.0^{d}	0.8±0.0^{d}	6.3±0.1^{d}	1.2±0.1^{d}	2.5±0.1^{d}	4.6±0.1^{c}	16.5^{d}
White cabbage	S	0.3±0.0^{c}	n.d.	1.8±0.0^{e}	n.d.	0.4±0.0^{a}	n.d.	2.5^{e}
	R	0.1±0.1^{c}	0.9±0.0^{e}	3.9±0.1^{e}	0.1±0.0^{e}	0.1±0.0^{e}	0.5±0.2^{d}	5.5^{a}

goitrin was not detected in the shoot. In all cultivars gluconasturtiin was the glucosinolate found in the highest contents in the roots. The content in the roots of curly kale was 2.3 times higher than that in pointed cabbage, while the roots of savoy cabbage had 1.6 times the amount of the roots of white cabbage.

Calculation of the total glucosinolate content per class is presented in Fig. 2A,B for roots and shoot respectively. Aromatic glucosinolates were highest in the roots of curly kale, accounting for 63% of the total content. The highest aliphatic glucosinolate content was observed in savoy cabbage, representing 50% of the total content. In aliphatic glucosinolates the S:N atom ratio is 2:1 (BLAKE-KALFF & al. 1998). It is worth to point out that savoy cabbage had the lowest sulfur content but the highest nitrogen content (Fig. 1A,B). The highest root indolyl glucosinolate content was found in curly kale, representing 21% of the total, whereas white cabbage had the lowest indolyl glucosinolate content, accounting for only 3% of the total glucosinolate content. In the shoot the glucosinolate content per class was generally lower than in the roots for all the cultivars. Furthermore, in the shoot of curly kale, red cabbage and white cabbage no aromatic glucosinolates were detected. The exception is savoy cabbage, in which aliphatic glucosinolates accounted for 43% of the total content.

Analysis of the amino acid pool (unpublished results) showed differences in some of the precursors between cultivars and plant parts. Methionine, the precursor for the aliphatic class, had a content of 0.9–5.3 μmol g^{-1} DW in the roots of white cabbage and curly kale, respectively. In curly kale methionine content in the shoot was 3.5 fold lower than in the roots, while the content of this amino acid in the shoot of savoy cabbage (1.6 μmol g^{-1} DW) was highest. Phenylalanine, the precursor for aromatic glucosinolates had a content of 0.7–1.9 μmol g^{-1} DW in the roots of savoy cabbage and curly kale, whereas the shoot of pointed cabbage had the lowest value (0.5 μmol g^{-1} DW) and savoy cabbage the highest value (1.6 μmol g^{-1} DW). Based on these results it seems unlikely that lack of precursors for glucosinolate biosynthesis for the various classes affected the results.

In all *Brassica* cultivars the total glucosinolate content was highest in the roots (Table 2). This is in agreement with findings with other species as *Arabidopsis thaliana* (GRASER & al. 2001) and other cultivars of *Brassica oleracea* (for detailed review see ROSA 1999, WESTERMAN & al. 2001). The cultivar with the highest glucosinolate content, savoy cabbage, had 1.4-fold less total glucosinolates, when calculated on a whole plant basis (after DE KOK & al. 2000), than found by KUSHAD & al. 1999 for different cultivars of *Brassica*. This difference might be explained by the different methodology used in glucosinolate extraction as well as a different quantification of the glucosinolate content.

Glucosinolate content in relation to the sulfur pool

How to express the glucosinolate content, either as fraction of the total sulfur or as organic sulfur fraction (Fig. 3A,B) is a question that can be raised. Generally, it is common to find the glucosinolate content expressed as a fraction of total sulfur content (WESTERMAN & al. 2001). Glucosinolate content expressed on a total sulfur basis ranged from 1–2%, for white cabbage and savoy cabbage (Fig. 3A,B). Calculation of the these data on an organic sulfur basis showed much higher values, *viz.* 10 and 23% for white cabbage and savoy cabbage, respectively. Expression of the glucosinolate fraction on an organic sulfur basis, therefore, might be more meaningful for *Brassica*, in view of the high sulfate content. Moreover, these secondary compounds are incorporated into the organic fraction, which also appears to be the best parameter to express the sulfur requirement for growth (DE KOK & al. 2002, CASTRO & al. 2003).

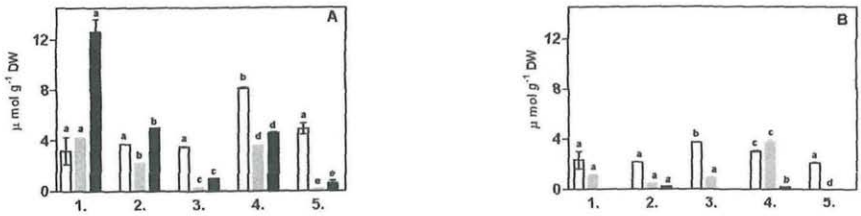

Fig. 2. Total glucosinolate content per class in roots (**A**) and shoot (**B**) in 5 cultivars of *Brassica oleracea*. 1. Curly kale, 2. Pointed cabbage, 3. Red cabbage, 4. Savoy cabbage, 5. White cabbage. Aliphatic, indolyl and aromatic glucosinolates are represented in light, grey and dark bars, respectively. Data represents the mean of 3 measurements with 3 plants in each (± SD). Means followed by the same letter are not statistically different at $P < 0.01$.

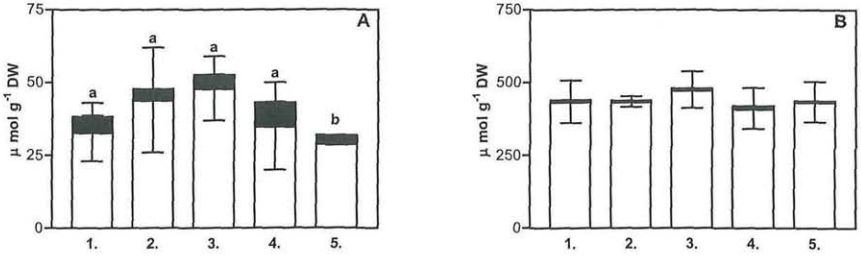

Fig. 3. Total glucosinolate content in 5 cultivars of *Brassica oleracea* expressed as a fraction of organic (A) and total (B) sulfur contents. Glucosinolate content is expressed in dark (■) and organic (a) and total sulfur (**b**) fraction in light (□) bars. 1. Curly kale, 2. Pointed cabbage, 3. Red cabbage, 4. Savoy cabbage, 5. White cabbage. Data represents the mean of 3 measurements with 3 plants in each (± SD). No significant differences were observed. Means followed by the same letter are not statistically different at $P < 0.01$.

In conclusion, the present results showed that the glucosinolates in shoot and roots of *Brassica oleracea* may represent a major proportion of the organic sulfur fraction. Whether these compounds play a role under conditions of sulfur and nitrogen deprivation or surplus needs further evaluation. This can be done by exposing plants to atmospheric H_2S and NH_3, in combination with various levels of pedospheric sulfur and nitrogen.

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