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G. Verlinden[a]; B. Pycke[a]; J. Mertens[b]; F. Debersaques[a]; K. Verheyen[c]; G. Baert[a]; J. Bries[b]; G. Haesaert[a]
[a] Department of Biosciences and Landscape Architecture, University College Ghent, Ghent, Belgium [b] Soil Service of Belgium, Heverlee, Belgium [c] Department of Forest and Water Management, University of Ghent, Gontrode, Belgium

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Application of Humic Substances Results in Consistent Increases in Crop Yield and Nutrient Uptake

G. Verlinden,[1] B. Pycke,[1] J. Mertens,[2] F. Debersaques,[1] K. Verheyen,[3] G. Baert,[1] J. Bries,[2] and G. Haesaert[1]

[1]Department of Biosciences and Landscape Architecture, University College Ghent, Ghent, Belgium
[2]Soil Service of Belgium, Heverlee, Belgium
[3]Department of Forest and Water Management, University of Ghent, Gontrode, Belgium

ABSTRACT

The effect of humic substances on yield and nutrient uptake of grass, maize, potato and spinach was investigated in six field experiments and two pot experiments in a high input cropping system. The humic substances originating from leonardite formations in Canada were applied as liquid solution to the soil (Humifirst liquid) or as a solid incorporated in mineral fertilizers (Humifirst incorporated). Formal meta-analysis of the results of all executed experiments showed that the application of humic substances had an overall positive effect on dry matter yield of the crops and this effect was statistically significant for Humifirst incorporated. In the case of permanent grassland, humic substances promoted mainly the production of the first grass cut, which has the highest grass quality among all cuts during the growing season. Tuber production on the potato field trial showed a high response on the application of humic substances. Total potato yield increased with 13 and 17% for Humifirst liquid and Humifirst incorporated, respectively. The effect of humic substances on maize yield was limited, probably due to the rather high nutrient status of both soils. Finally, the formal meta-analysis showed a consequent increase in nitrogen and phosphorus uptake of all studied crops as well. The effect on potassium and magnesium uptake was also mainly positive, while sodium and calcium uptake were not affected in most of the experiments.

Keywords: humic substances, leonardite, macronutrients, grass, maize, potato, spinach

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Address correspondence to G. Verlinden, University College Ghent, Voskenslaan 270, B-9000 Ghent, Belgium. E-mail: greet.verlinden@hogent.be

INTRODUCTION

High input cropping systems with application of inorganic fertilizers or slurry as organic fertilizer are often faced with decreasing soil organic matter levels. In Flanders (the northern part of Belgium), the organic matter content of more than 30% of the arable land is lower than the optimal value defined by the Soil Service of Belgium (Sleutel et al., 2003; Vanden Auweele et al., 2004). In many other European regions with comparable agricultural activity a similar evolution occurs (Vleeshouwers and Verhagen, 2002). As soil organic matter is crucial for optimal soil quality and soil fertility, large amounts of external organic sources are needed to improve the organic matter status of the soil. However, application of high quantities of nitrogen- (N) and phosphorous- (P) rich organic materials comes into conflict with the strict nutrient legislations in most European countries.

An alternative solution is the application of humic substances to the soil. Humic substances are produced by the decay of organic materials and are found in soil, peat and leonardite (Stevenson, 1994). Humic substances can be divided into humic acids, fulvic acids, and humins based on their solubility in acid and alkali (Schnitzer, 1986). Many of the beneficial characteristics of soil organic matter are associated with humic substances which are recognized as the most chemically active compounds in soils, with cation and anion exchange capacities far exceeding those of clays (Stevenson, 1994; Koopal et al., 2005).

The positive effect of humic substances on the growth of numerous plants is well documented (Visser, 1986; Chen and Aviad, 1990). Several authors demonstrated that the addition of particular concentrations of humic substances can favor the growth of both the root and the aerial parts of the plant and encourage nutrient absorption. Ayuso et al. (1996) investigated the effect of humic substances originating from various organic materials on the growth and nutrient absorption of barley during hydroponic cultivation. They found that doses representing less than 10 mg L^{-1} carbon favored plant growth, while higher doses sometimes inhibited it. The absorption of macronutrients was significantly affected by the addition of humic substances but differed for each nutrient. Sharif et al. (2002) sprayed 50 to 300 mg kg^{-1} humic acids on the soil in a pot experiment with maize and found that the addition of 50 and 100 mg kg^{-1} caused a significant increase of 20 and 23% in shoot and 39 and 32% in root dry weight. Plant N accumulation was increased significantly over control whereas plant P accumulation did not increase significantly. Tufencki et al. (2006) applied increasing doses of humic acids, varying from 500 to 2000 mg kg^{-1}, at different times before lettuce seedling transplantation, to experimental soil placed in pots. Especially early application of humic acids had positive impacts on the plant growth and nutrient contents of lettuce plants with a short growing period. Fernandez-Escobar et al. (1996) studied the effect of foliar application of leonardite extracts to young olive plants in greenhouse and in field experiments. Under field conditions, shoot growth and accumulation of potassium (K), boron (B), magnesium (Mg), calcium (Ca), and iron (Fe) in leaves was promoted.

The effects of humic substances on plant production and nutrient absorbance generally depends on their origin, type and concentration and on the species and variety of the plant treated (Visser, 1986; Chen and Aviad, 1990). Experimental conditions are very important as well. Most of the cited studies are executed in greenhouse or laboratory conditions and, to our knowledge, the effect of humic substances in field conditions has rarely been reported before in scientific papers. Hence, the main purpose of this work was to determine the potential of humic substances to affect yield and nutrient uptake of maize, grass, and potato in field experiments. To allow joined conclusions throughout the different experiments, the overall effect of humic substances for all experiments was analyzed by means of a formal meta-analysis following Gurevitch and Hedges (2001). This method consists of integrating findings of independent studies by calculating the magnitude of treatment effects (effect size).

MATERIALS AND METHODS

Humic Substances

A liquid mixture of humic and fulvic acids (Humifirst®) was used as organic amendment. The mixture contained 12% humic and 3% fulvic acids (weight/weight solution), further indicated as Humifirst liquid. Humifirst liquid was sprayed on the soil before seed bed tillage or on the plants early in the growing season. Besides the liquid form, also the solid form Humifirst WG, containing 68% humic substances, was used in the experiments. Humifirst WG was incorporated into mineral fertilizers at varying concentrations and will be further referred to as Humifirst incorporated. The humic substances in both products were extracted from Canadian leonardite which is highly oxidized lignite with more than 85% humic acids.

Data Collection

Six field experiments conducted in 2006 were included in the study. Additionally, two preceding pot experiments were included as well. For all experiments, the experimental design was a randomized complete block with four replications in the field experiments and five to seven replications in the pot experiments. The field experiments were all located in Flanders (Belgium) on different soil types. The location and the characteristics of the soils are shown in Table 1. The crops studied in the field experiments were grass (trial G1, G2, and G3), maize (trial M1 and M2) and potato (trial P1) and additionally grass (experiment PotG) and spinach (experiment PotS) were studied in the

Table 1
Characteristics of the experimental soils

Trial	Soil type	Location	Organic matter % carbon[1]	pH-KCl[2]	mg $100g^{-1}$ dry soil[3] P	K	Mg	Ca	Na
G1	Loamy sand	Bottelare	1.1	5.4	23	8	14	59	1.2
G2	Loamy sand	Bottelare	0.8	5.2	13	8	6	67	1.3
G3	Sand	Hoogstraten	1.8	4.9	44	14	9	88	2.4
M1	Loam	Moortsele	0.9	6.2	29	21	13	109	1.0
M2	Sandy loam	St-Niklaas	0.9	6.2	49	25	20	123	1.2
P1	Sandy loam	Moortsele	0.6	4.9	25	15	12	70	1.3
PotG-PotS	Sandy loam		0.9	5.2	33	20	14	78	2.1

[1]Organic carbon measured with a modified Walkley and Black method.
[2]pH determined in a soil-solution ratio of 1:1 (1 M potassium chloride).
[3]Measured in ammonia-lactate extract.

preceding pot experiments. For each experiment three comparable treatments were selected for further analysis: i) control treatment with mineral fertilization according to fertilization recommendations based on chemical soil analysis, ii) mineral fertilization plus 50 L ha^{-1} Humifirst liquid, and iii) mineral fertilization with Humifirst incorporated. An overview of the treatments with the amount of humic substances applied is given in Table 2. Plant parameters used in this study are i) dry matter yield (except for the pot experiment with spinach where fresh yield was measured) and ii) uptake of macronutrients N, P, K, Mg, Na, and Ca by the plants.

For the grassland field trials, the number of grass cuts depended on the weather and grass conditions on each field (between two and four cuts). Fresh yield was determined by cutting a subplot of 13 m^2 and a representative grass sample was taken per replication to measure dry matter content and mineral composition. Field experiment G2 was sown in April 2006 and the first grass cut was only taken at the end of June 2006. As a result, data of 2006 were not comparable to the results of the permanent grass fields G1 and G3. Therefore, field experiment G2 was also studied in 2007 and these data were used in the formal meta-analysis. Harvest on the maize fields was executed at the end of September (M2, 28th of September) or in the beginning of October (M1, 5th of October). Fresh weights of the cobs and the green parts of the maize (aboveground biomass) were measured for each replication and representative samples were taken to analyze dry matter content and mineral composition. On the potato field, potato tubers of each replication were harvested in the middle of September, weighed, and sorted into different size classes (<32 mm, 32–45 mm, and >45 mm). A sample of the size >45 mm was transported to the

Table 2
Treatments on the experimental fields

Trial	Crop	Soil type	Treatments: 1. Control	2. Humifirst liquid	Amount HS kg ha^{-1}	3. Humifirst incorporated	Amount HS kg ha^{-1}	Number of plots	Experim. unit (m^2)
			Field Experiments						
G1	Permanent grassland	Loamy sand	Mineral fertilization	Mineral fertilization + 50 L ha^{-1} HF	8.25	Mineral fertilization + 3.5%	14.88	12	45
G2	New grassland	Loamy sand	Mineral fertilization	Mineral fertilization + 50 L ha^{-1} HF	8.25	Mineral fertilization + 1.5%	3.57	12	45
G3	Permanent grassland	Sand	Mineral fertilization			Mineral fertilization + 3.5%	5.65	8	102
M1	Maize	Loam	Mineral fertilization widespread	Mineral fertilization widespread + 50 L ha^{-1} HF	8.25			8	60
			Row fertilization			Row fertilization + 1.5%	1.50	8	60
M2	Maize	Sandy loam	Mineral fertilization widespread	Mineral fertilization widespread + 50 L ha^{-1} HF	8.25			8	60
			Row fertilization			Row fertilization + 3.5%	3.57	8	60
P1	Potato	Sandy loam	Mineral fertilization	Mineral fertilization + 50 L ha^{-1} HF	8.25	Mineral fertilization + 0.5%	3.89	12	54
			Pot Experiments						
PotG	Grass	Sandy loam	Mineral fertilization	Mineral fertilization + 50 L ha^{-1} HF	8.25	Mineral fertilization + 1.5%	4.10	12	1*L*
PotS	Spinach	Sandy loam	Mineral fertilization	Mineral fertilization + 50 L ha^{-1} HF	8.25	Mineral fertilization + 1.5%	4.10	12	1*L*

HS = Humic substances; HF = Humifirst.

As field G2 was a new grassland in 2006, the same treatments were applied again in 2007. The first cut in 2007 was taken just after the winter period before fertilization and Humifirst application in 2007.

laboratory to measure the concentration of mineral elements in the potatoes. The pot experiments were carried out in 1 L capacity containers, each containing 1 kg of experimental soil and 1 g of grass seed (Italian Ryegrass) or three spinach seedlings. The pots were placed in the growth chamber with day/night temperatures of 22°C/14°C and a relative humidity of 80%. The grass was cut three times, fresh and dry yield was measured and mineral composition of the grass was analyzed. Spinach was harvested two months after sowing, fresh weight was measured, and mineral composition of the dried plant samples was determined.

Data Analysis

A distinction was made between the effects of Humifirst liquid and Humifirst incorporated. First, the change in dry matter yield and nutrient uptake due to the application of Humifirst as the percentage of the value in the control was calculated for each experiment (Tables 3 and 4). The difference in yield and nutrient uptake between the control and the Humifirst treatments was statistically tested with an analysis of variance in combination with a Tukey test (SAS Package, Version 4.1, SAS Institute Inc., Cary, NC, USA).

To compare the results between the experiments, a formal meta-analysis was performed following Gurevitch and Hedges (2001). Meta-analysis requires three basic statistics of both the control and experimental group: number of replicates, mean, and standard deviation. Analysis was done separately for yield and nutrient uptake of each macronutrient both for Humifirst liquid and Humifirst incorporated. For the grassland field trials, the formal meta-analysis was performed on the experimental results of the first grass cut and of the total growing season. As the first grass cut is most important in grassland exploitation through its high grass quality, only the results of this meta-analysis are reported for the grassland experiments.

The experiments were assembled into four classes (index i in formulas): "grassland field studies", "maize field studies", "potato field studies" and "pot experiments". For each experiment (index j in formulas), the unbiased standardized mean difference "d" was calculated as the difference between the means of the above-ground biomass of the experimental and control group ($\bar{X}_{ij}^{H} - \bar{X}_{ij}^{C}$), standardized by their pooled standard deviation (s_{ij}) and corrected for bias due to small sample sizes (J) (Equation 1). In the experiments discussed here, the experimental group represents the Humifirst treatment and a positive effect size indicates a positive response of crop yield or nutrient uptake on the application of Humifirst (liquid or incorporated).

$$d_{ij} = \frac{\bar{X}_{ij}^{H} - \bar{X}_{ij}^{C}}{s_{ij}} J \tag{1}$$

Table 3

Dry matter yield (DM, control in tons ha^{-1}) and nutrient uptake (N, P, K, Mg, Na and Ca, control in kg ha^{-1}) of the different grass cuts on the grass field trials and of the maize and potato trials (Humifirst treatments expressed as % of control)

	DM yield			N-uptake			P_2O_5-uptake[1]			K_2O-uptake[1]			MgO-uptake[1]			Na_2O-uptake[1]			CaO-uptake[1]		
	Control tons ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %	Control kg ha^{-1}	HF liq %	HF inc %
Gl																					
C1	3.4	112	103	80	108	108	26	101	111	81	110	111	10	103	109	9	98	106	19	101	106
C2	5.7	94	89	107	103	101	34	91	91	132	88	90	19	92	92	12	88	96	37	95	87
Total	9.4	98	92	187	105	104	60	95	100	213	96	98	29	96	98	21	92	100	56	97	93
G2_2006																					
C1	3.7	96	85	100	101	93	25	96	88	153	100	93	11	106	91	4	130	106	23	109	107
C2	2.1	104	91	67	98	81*	11	110	104	95	94	94	12	104	81	3	106	80	25	99	76
C3	2.8	107	107	100	102	98	19	107	101	128	108	109	11	114	100	5	123	135	25	112	99
Total	9.3	101	93	267	101	92	55	103	96	375	101	98	33	108	90	12	122	112	73	107	94
G2_2007																					
C1	1.8	116	103	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
C2	3.6	101	98	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
C3	3.9	91	97	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total	9.3	99	99	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
G3																					
C1	2.5		142**	57		172**	20		184***	66		197**	8		150**	3		201***	16		142**
C2	2.2		87	56		83	21		82	84		78	8		87	3		98	13		88
C3	1.7		86	34		96	14		89	66		92	8		86	2		81	14		85
C4	1.7		114	47		113	22		106	83		107	8		104	3		88	13		101
Total	8.1		110	195		119	77		116	299		116	33		107	11		116	56		106
Ml																					
Widespread fert	16.9	102		226	100		69	116		312	93		40	105		5	110		43	93	
Row fert.	17.4		103	225		108	74		110	301		108	47		103	4		97	55		92
M2																					
Widespread fert.	18.6	100		256	104		180	107		328	99		67	102		6	83		47	85	
Row fert.	18.8		101	268		100	204		98	351		111	74		96	6		101	50		81
PI	51.2	113	117	154	111	124	55	154	160	268	114	113	17	123	125	2	143	125	6	97	94

[1]The uptake of P, K, Mg, Na and Ca expressed as the uptake of P_2O_5, K_2O, MgO, Na_2O and CaO in kg ha^{-1}.

C1, C2, C3, C4: number of grass cuts; HF liq : Humifirst liquid, HF inc : Humifirst incorporated; fert.: fertilization.

*, **, ***: significant difference between Humifirst and control treatment with respectively $p < 0.05$, $p < 0.01$, $p < 0.001$ (Tukey test).

Table 4

Dry matter yield (control in g pot^{-1}) and nutrient uptake (N, P, K, Mg, Na and Ca, control in mg pot^{-1}) of the different grass cuts in the grass pot experiment and fresh yield (control in g pot^{-1}) and nutrient uptake (N, P, K, Mg, Na and Ca, control in mg pot^{-1}) of the spinach pot experiment (Humifirst treatments expressed as % of control)

	Yield			N-uptake			P_2O_5-uptake[1]			K_2O-uptake[1]			MgO-uptake[1]			Na_2O-uptake[1]			CaO-uptake[1]		
	Control g pof^{-1}	HF liq %	HF inc %	Control mg pof^{-1}	HF liq %	HF inc %	Control mg pof^{-1}	HF liq %	HF inc %	Control mg pof^{-1}	HF liq %	HF inc %	Control mg pof^{-1}	HF liq %	HFinc %	Control mg pof^{-1}	HF liq %	HF inc %	Control mg pof^{-1}	HF liq %	HF inc %
Pot_G																					
Cl	1.5	106	107	84	110	110	13	120	114	112	112	109	10	107	104	3	112	92	15	99	101
C2	1.4	92	89	64	90	103	12	118	105	76	108	97	9	127	114	4	140	91	10	106	107
C3	1.0	126	113	50	137	160	9	136	117	46	131	129	9	143	113	3	168	116	11	144	119
Total	3.9	106	102	198	111	120	34	123	112	234	114	109	28	124	110	9	139	98	35	114	108
Pot_S	7.3	110	113	—	—	—	42	136	116	303	95	93	35	110	91	12	119	67	25	106	85

[1]The uptake of P, K, Mg, Na and Ca expressed as the uptake of P_2O_5, K_2O, MgO, Na_2O, and CaO in mg pot^{-1}.

C1, C2, C3: number of grass cuts; HF liq : Humifirst liquid, HF inc : Humifirst incorporated.

No significant differences between Humifirst and control treatments.

where

$$s_{ij} = \sqrt{\frac{\left(N_{ij}^{H} - 1\right)\left(s_{ij}^{H}\right)^2 + \left(N_{ij}^{C} - 1\right)\left(s_{ij}^{C}\right)^2}{N_{ij}^{H} + N_{ij}^{C} - 2}} \tag{2}$$

$$J = 1 - \frac{3}{4\left(N_{ij}^{H} + N_{ij}^{C} - 2\right) - 1} \tag{3}$$

$$v_{ij} = \frac{N_{ij}^{H} + N_{ij}^{C}}{N_{ij}^{H} N_{ij}^{C}} + \frac{d_{ij}^{2}}{2\left(N_{ij}^{H} + N_{ij}^{C}\right)} \tag{4}$$

where N_{ij}^{C} = total number of replications in the control group, N_{ij}^{H} = total number of replications in the experimental group, s_{ij}^{C} = standard deviation of the replications in the control group, s_{ij}^{H} = standard deviation of the replications in the experimental group, v_{ij} = the variance in the effect for the jth experiment in the ith class. With this parameter, confidence intervals can be calculated. As the sample size increases, J approaches 1.

In order to calculate means and accompanying confidence intervals for each class, their mean effect sizes were combined with the fixed effect model (Gurevitch and Hedges, 2001). It was thus assumed that the experiments within each of the classes share a common true effect size. The cumulated mean effect size within the ith class "d_{i+}" is a weighed average of the effect size estimates for the experiments in that class. Thus larger experiments, which are assumed to yield more precise results, are given more weight.

$$d_{i+} = \frac{\sum_{j=1}^{k_i} w_{ij} d_{ij}}{\sum_{j=1}^{k_i} w_{ij}} \tag{5}$$

$$s^2(d_{i+}) = \frac{1}{\sum_{j=1}^{k_i} w_{ij}} \tag{6}$$

with $w_{ij} = 1/v_{ij}$ as weight for the jth experiment in the ith class and where s^2 is the variance of d_{i+}. With this variance, the 95% confidence interval for d_{i+} can be calculated.

For the evaluation of the overall effect of Humifirst liquid and incorporated on yield and nutrient uptake, the grand mean effect size across the 4 classes "d_{++}" was calculated.

$$d_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij} d_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij}} \tag{7}$$

$$s^2(d_{++}) = \frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k_i} w_{ij}} \tag{8}$$

where m is the total number of classes (m = 4, grass, maize, potato and pot experiments).

The grand mean effect sizes on yield and nutrient uptake (N, P, K, Mg, Na, and Ca) are further indicated with $d_{++\text{yield}}$ and $d_{++\text{Nup}}$, $d_{++\text{Pup}}$, $d_{++\text{Kup}}$, $d_{++\text{Mgup}}$, $d_{++\text{Naup}}$ and $d_{++\text{Caup}}$. Cohen (1969) provides a conventional interpretation of the magnitude of effect sizes: 0.2 is a 'small' effect, 0.5 is 'medium' in magnitude, 0.8 is 'large', and any effect greater than 1.0 would be 'very large'.

RESULTS AND DISCUSSION

Influence of Humic Substances on Crop Yield

A general increase in yield of the studied crops was observed by application of humic substances (Figure 1). The grand mean effect size ($d_{++\text{yield}}$) of humic

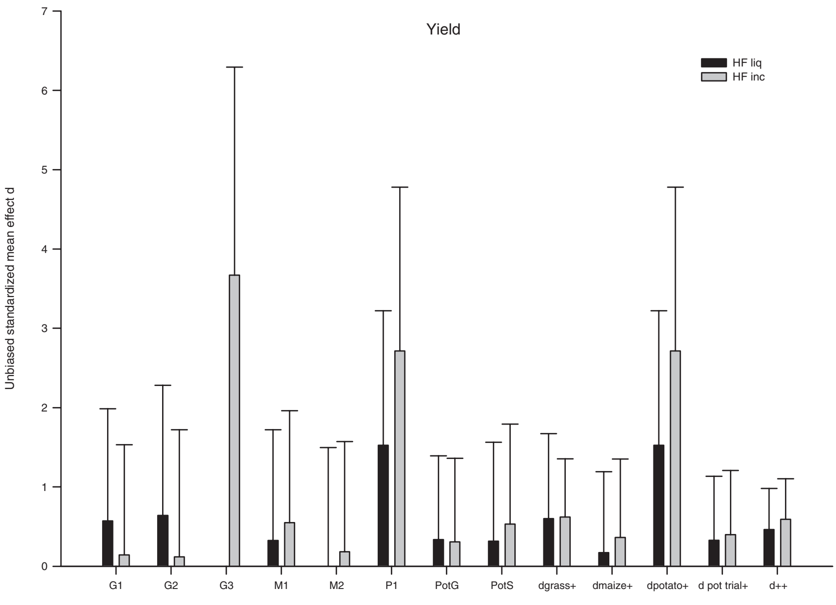


Figure 1. Meta-analysis of yield in field and pot experiments. Mean effect of Humifirst liquid (black bars) and Humifirst incorporated (grey bars) on crop yield, expressed as the unbiased mean effects (d) for the grassland trials (G1, G2 and G3), the maize trials (M1 and M2), the potato trial (P1) and the pot experiments (PotG and PotS). When d is positive, yield is increased by the Humifirst treatment. d_{++} is the grand mean effect size across the 8 studies, $d_{\text{grass}+}$ the cumulated mean effect size for the grass field trials, $d_{\text{maize}+}$ for the maize field trial and $d_{\text{pottrial}+}$ for the pot experiments. The upper limit of the 95 % confidence interval is indicated by the error bar.

substances on yield of all studied crops is positive, with $d_{++\text{yield}}$ equal to + 0.46 with 95% confidence interval [−0.06; + 0.98] for the Humifirst liquid treatment and d_{++} equal to + 0.59 with 95% confidence interval [+ 0.08; + 1.10] for the Humifirst incorporated treatment (black and white bars in Figure 1, respectively). Thus, the largest and statistically significant effect was measured for the incorporated humic substances.

Variation between the replicates of the individual experiments was quite high. Therefore, the observed positive effects of humic substances on yield in the different experiments could not be statically approved (except for incorporated Humifirst in the potato field experiment, see below).

Table 3 presents an overview of the individual effects of humic substances on grass, maize, and potato yield in the field experiments. On the permanent grassland of G1 and G3 and in the second year of the newly sown grassland G2 application of humic substances in combination with mineral fertilization resulted in a positive dry matter yield response at the first grass cut. Humifirst liquid increased grass production with 12% (G1) and 16% (G2) compared to the control and Humifirst incorporated increased the production at the first cut with 3% (G1 and G2) and 42% (G3) (highly statistically significant). The higher grass production at the first grass cut was always followed by lower production levels in the next grass cuts. The decline in grass production after a high yielding first grass cut is a well-known phenomenon in grass physiology (Behaeghe, 1979). Stimulated by the humic substances, the grass uses a lot of its reserves for the production of the first grass cut so that regrowth is hampered by a lack of reserves in the roots. Even though this decline caused similar total yield levels at the end of the growing season for the treated and untreated plots in G1 and G2 (Table 3), the increase of grass production at the first cut is most important in grassland exploitation because of its high quality level. The grass quality was not decreased with yield increase after application of humic substances (data not shown).

For G2 in 2006, when the grass was just sown, the effect of humic substances did not correspond with the general trend on grassland as described above. At the first grass cut, grass production was similar on the control plots and the Humifirst plots. Only later in the growing season, at the second and especially at the third grass cut, the positive effect of humic substances on grass production was expressed.

Less optimal growing conditions are likely to benefit the positive effect of humic substances. The third grass cut (2006) in field experiment G2 and the fourth grass cut in field experiment G3 were taken after a long dry period in July 2006 and for both grass cuts a clear yield increase was measured on both the Humifirst liquid and Humifirst incorporated treatment (plus 7 and 14% dry matter yield compared to control). These observations also indicate a long term effect of humic substances application. Due to the simple treatment of humic substances at the start of the growing season in 2006, a positive effect on dry matter yield could still be observed at the third grass cut on G2 and at the fourth grass cut on G3 in 2006 and even at the first grass cut of 2007 on G2.

From the results of the formal meta-analysis on the grassland data, a systematic but non-significant increase in yield at the first cut was assessed after application of Humifirst liquid and Humifirst incorporated. The cumulated mean effect size within the grassland class ($d_{\text{grass}+}$) is + 0.60 with 95% confidence interval [−0.47; 1.67] for the Humifirst liquid treatment and + 0.62 with 95% confidence interval [−0.11; 1.35] for the Humifirst incorporated treatment.

The application of humic substances in the two maize experiments only induced a small effect on the dry matter yield of field M1 (+ 2 to + 3% compared to control, Table 3) and hardly no effect on the dry matter yield of field M2 (−3 to + 3% compared to control). The formal meta-analysis also generated a small cumulated mean effect size within the maize class ($d_{\text{maize}+}$) of + 0.17 with 95% confidence interval [−0.85; 1.19] for the Humifirst liquid treatment and + 0.36 with 95% confidence interval [−0.63; 1.35] for the Humifirst incorporated treatment. These limited positive results could be related to the rather high nutrient status of the soils. Especially field M2 had a high available nutrient reserve as shown in Table 1 (soil properties of M2) and as shown through the high relative dry matter yield of unfertilized control plots on the field (99% of the yield of fertilized control plots, data not shown in Table 4). On field M1, relative dry matter yield of unfertilized control plots amounted to 90% of the yield on the fertilized control plots. The high soil nutrient status of fields M1 and M2 was due to repeated slurry applications in the past.

On potato field P1 a high response of tuber production on the application of humic substances was observed (Table 3). Total potato yield increased with 13 and 17% compared to control for Humifirst liquid and Humifirst incorporated respectively. According to the meta-analysis a large standardized mean difference between the yield of the Humifirst treatments and the control was calculated: + 1.52 with 95% confidence interval [− 0.17; + 3.22] for the Humifirst liquid and + 2.71 with 95% confidence interval [+ 0.65; + 4.78] for the Humifirst incorporated treatment.

The field experiments confirmed the positive results which were observed in the two preceding pot experiments. For both the grass and spinach pot experiment a positive effect on yield was measured. Dry matter grass production (total of three grass cuts) was increased with 6 and 2%, respectively, for Humifirst liquid and Humifirst incorporated (Table 4). At the first grass cut, grass production increased with 6 and 7 % by application of the two Humifirst products. The unbiased standardized mean difference between the yield of the Humifirst treatments and the control at the first grass cut was + 0.34 with 95 % confidence interval [−0.72; + 1.39] and + 0.31 with 95% confidence interval [−0.75; + 1.36] for the Humifirst liquid and the Humifirst incorporated treatment, respectively (Figure 1). The fresh yield of spinach leaves increased with 10 and 13% after application of Humifirst liquid and incorporated. The respective standardized mean differences are + 0.32 with 95% confidence interval [−0.93; + 1.56] and + 0.31 with 95% confidence interval [−0.73; + 1.79].

The growth promoting results of humic substances are in agreement with those reported for a wide number of plant species (Visser, 1986; Chen and Aviad, 1990). The good results of the potato field trial correspond with the conclusions of a study in 2005 from the Potato Research Institute in Finland (Kuisma, 2005). In this study Humifirst also had a positive effect on total tuber yield (+ 17% compared to control) and marketable yield (+ 24% compared to control). The best response was obtained when Humifirst was applied to the soil just before seed bed tillage, which is similar to our experiment, compared to later application on planting and hilling. Other positive effects of Humifirst on potato yield were found at Gembloux (+ 25%) and Geer (+ 11%) both located in the southern part of Belgium (Anonymous, 2002). Eyheraguibel (2004) detected that humic substances accelerated both vegetative and reproductive growth of maize plants and thus stimulated optimal production of plant biomass (shoot and cobs). Root growth was stimulated as well with more fine lateral and secondary roots in the humic substances treatments. In line with these results Sharif et al. (2002) reported a yield increase of 20 to 23% in shoot dry weight and 32 to 39% in root dry weight of maize in a pot experiment. The incorporation of humic substances in the soil stimulated root mass of creeping bentgrass with 45% in the 0 to 10 cm depth and with 38% in the 10 to 20 cm depth (Cooper et al., 1998). Aboveground biomass was only slightly promoted and was attributed by the authors to a sufficient nutrient supply. This observation seems to correspond with the limited effects on our maize fields M1 and M2 with a rather high nutrient status of the soil.

The growth promoting effects of humic substances are optimal when applied in limited amounts. Ayuso et al. (1996), Sharif et al. (2002) and Pilanah and Kaplan (2003) did several experiments with increasing doses of humic substances. Best results were obtained with amounts of order of 4 to 30 kg humic substances ha^{-1}, which correspond with the doses used in our experiments (Table 2).

Influence of Humic Substances on Nutrient Uptake

The amount of nutrient uptake by the plants in the control plots (in kg ha^{-1} for field experiments and in mg pot^{-1} for pot experiments) and the change by the application of humic substances (in percentage to control) are presented in Table 3 and 4.

With the formal meta-analysis on the experimental results, a systematic increase in nitrogen uptake by application of humic substances was observed except for one case (Figure 2a, experiment M2, Humifirst incorporated). The grand mean effect size of humic substances on nitrogen uptake ($d_{++\mathrm{Nup}}$) is + 0.54 with 95% confidence interval [−0.07; + 1.14] for Humifirst liquid and + 0.78 with 95% confidence interval [+ 0.08; + 1.10] for Humifirst incorporated.

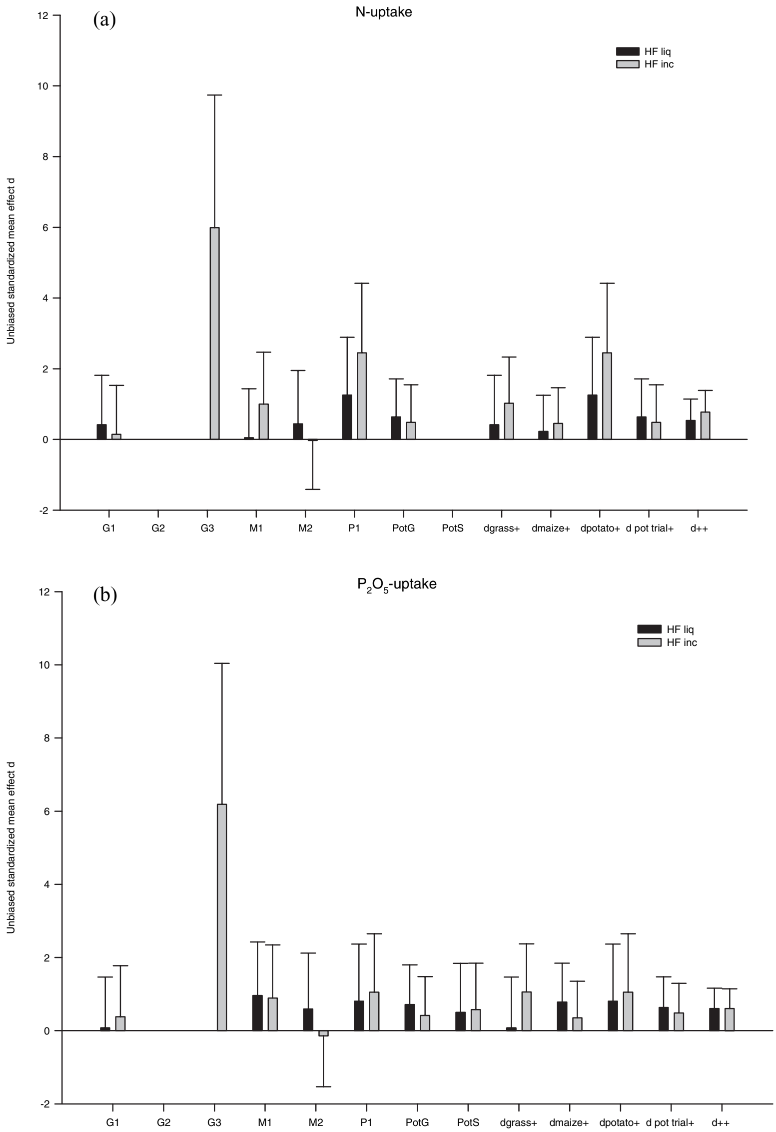
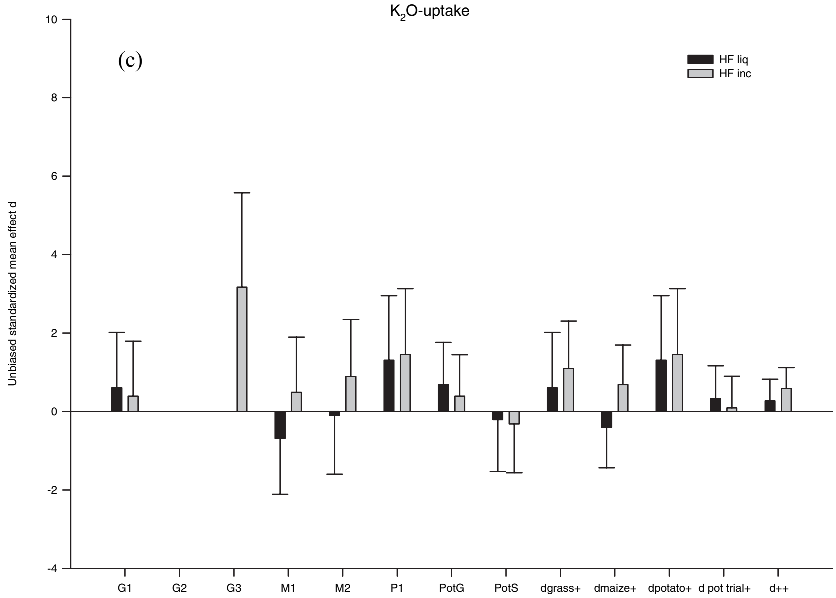


Figure 2. Meta-analysis of nutrient uptake (N, P, K, Mg, Ca and Na) in field and pot experiments. Mean effect of Humifirst liquid (black bars) and Humifirst incorporated (grey bars) on N-uptake (Fig2a), P_2O_5-uptake (Fig2b), K_2O-uptake (Fig2c), MgO-uptake (Fig2d), CaO-uptake (Fig2e) and Na_2O-uptake (Fig2f), expressed as the unbiased mean effects (d) for the grassland trials (G1, G2 and G3), the maize trials (M1 and M2), the potato trial (P1) and the pot experiments (PotG and PotS). When d is positive, yield is increased by the Humifirst treatment. d_{++} is the grand mean effect size across the 8 studies, d_{grass+} the cumulated mean effect size for the grass field trials, d_{maize+} for the maize field trial and $d_{pottrial+}$ for the pot experiments. The upper limit of the 95 % confidence interval is indicated by the error bar. *(Continued)*



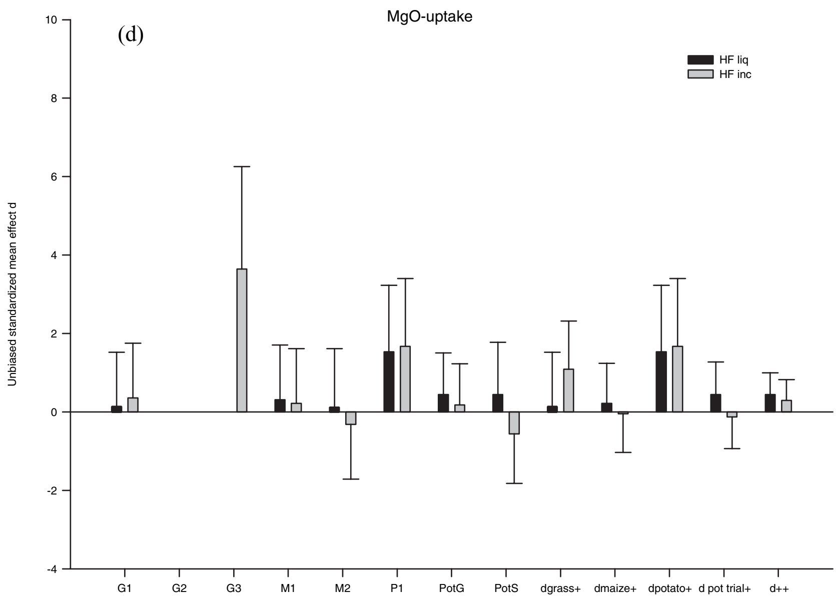


Figure 2. (Continued)

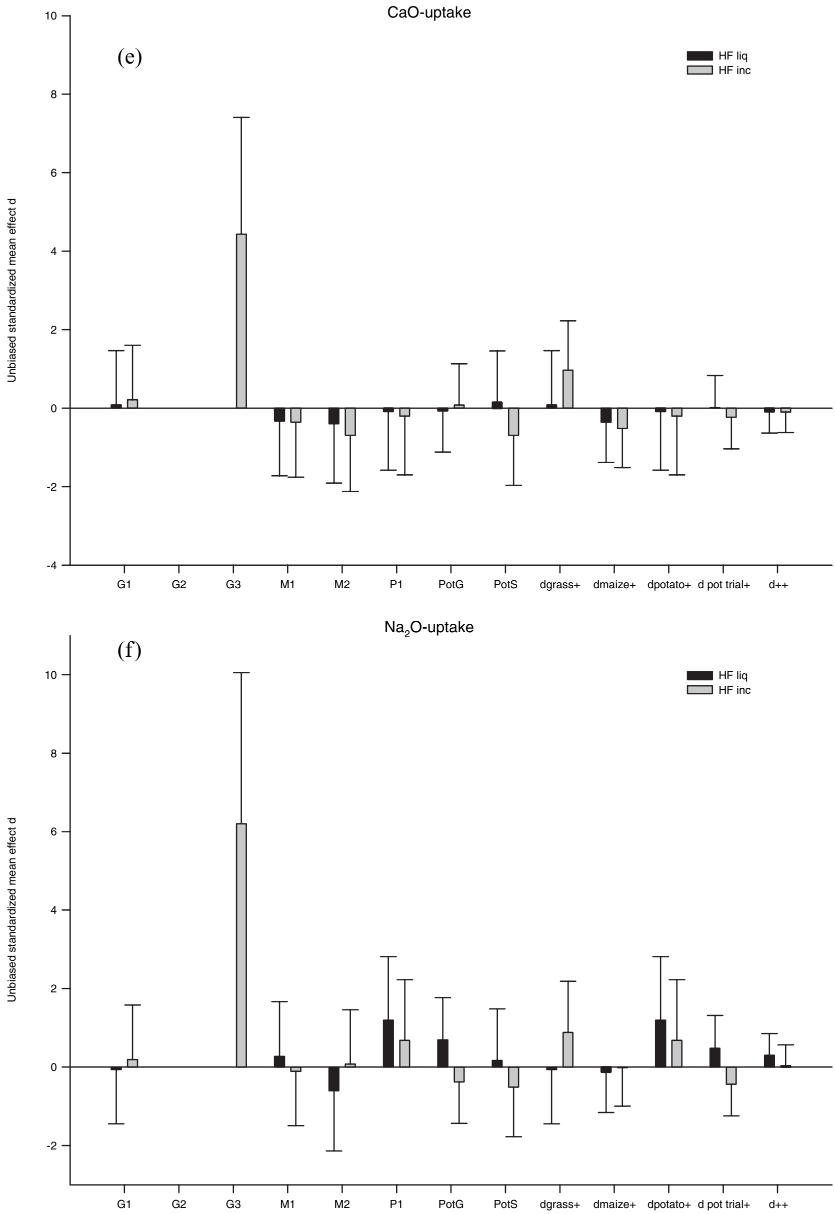


Figure 2. (Continued)

The effect of Humifirst incorporated on nitrogen uptake is larger than Humifirst liquid which may be due to the close contact between the fertilizer and the humic substances.

The uptake of phosphorus also increased in all experiments except one (experiment M2, Humifirst incorporated). Figure 2b shows a similar and consequent effect in all experiments. The grand mean effect size on phosphorus uptake ($d_{++\text{Pup}}$) is + 0.61 for both the Humifirst liquid and incorporated with 95 % confidence interval [+ 0.05; + 1.16] and [+ 0.07; + 1.14], respectively.

Potassium uptake was significantly enhanced by the incorporated humic substances ($d_{++\text{Kup}} = 0.58$ with 95% confidence interval [+ 0.05; + 1.11]) but less by the liquid humic substances ($d_{++\text{Kup}} = 0.27$ with 95% confidence interval [− 0.29; + 0.82]). In three of the six relevant experiments Humifirst liquid had no or a negative effect on potassium uptake (Figure 2c).

Also for magnesium uptake (Figure 2d) the grand mean effect size of humic substances ($d_{++\text{Mgup}}$) is positive, namely + 0.44 with 95% confidence interval [− 0.11; + 1.00] for Humifirst liquid and + 0.29 with 95% confidence interval [− 0.24; + 0.82] for Humifirst incorporated. In this case, the effect of the incorporated humic substances was less pronounced.

Sodium and calcium uptake were not affected by the application of humic substances (Figures 2e and 2f). The grand mean effect size of Humifirst incorporated on sodium uptake ($d_{++\text{Naup}}$) is + 0.04 with 95% confidence interval [− 0.49; + 0.56] and the effect of both Humifirst liquid and incorporated on calcium uptake is − 0.10 with 95% confidence interval [− 0.63; + 0.44]. Only the application of Humifirst liquid did increase the overall sodium uptake slightly ($d_{++\text{Naup}} = 0.30$ with 95% confidence interval [− 0.25; + 0.86]).

The above results show that the uptake of the most important macronutrients was enhanced with application of humic substances. Increased uptake of macro- and micronutrients has been reported before when humic substances were applied to the soil as simple solution (Cooper et al., 1998; Sharif et al., 2002) and even more pronounced when they were mixed into the nutrient solution (Ayuso et al., 1996; Pinton et al., 1999), suggesting the existence of a synergistic effect of combined applications of mineral nutrients and humic substances. As humic substances behave like weak acid polyelectrolytes, the occurrence of anionic charged sites accounts for the ability to retain cations like K^+ and Mg^{2+} and the cation exchange capacity of the soil will be increased.

In our experiments nitrogen and phosphorous uptake were most affected by the application of humic substances. Pinton et al. (1999) suggest that humic substances play a role in the modulation of nitrate uptake via an interaction with plasma membrane H^+ -ATPase. In their study the contemporary presence of nitrate and humic substances caused stimulation of the nitrate uptake capacity and of the plasma membrane H^+ -ATPase activity with the same pattern observed for nitrate uptake. The stimulation of plasma membrane H^+ -ATPase activity was also reported by several other authors (Maggioni et al., 1987; Canellas et al., 2002) and is considered as an important action of humic substances on plant nutrient acquisition. The enhanced uptake of phosphorous in plants with application of humic substances is mainly due to the increased availability of phosphate in the soil (Burns et al., 1986; Zalba and Peinemann, 2002). In many soils a large part of total phosphorous is insoluble (calcium phosphate precipitation) and thus unavailable to the plants. The major mechanism involved in the effect of humic substances increasing phosphorus recovery is the interference on calcium phosphate precipitation (Delgado et al., 2002; Satisha and Devarajan, 2005). In general, humic substances application increases also root mass and root volume (Burns et al., 1986; Sharif et al., 2002; Canellas et al., 2002; Eyheraguibel, 2004), which is an important factor in nutrient uptake as well.

Given the importance of macronutrients for plant growth, it is not surprising that both crop yield and nutrient uptake were positively affected by the application of humic substances. The same link was reported by several other authors (Burns et al., 1986; Ayuso et al., 1996; Fernandez-Escobar, 1996; Pinton et al., 1999).

CONCLUSION

Application of humic substances at the start of the growing season induced an overall positive effect on dry matter yield in the field and pot experiments. The observed effects were largest for the potato field, followed by the grasslands and were smallest for the maize fields. Plant uptake of nitrogen, phosphorous, potassium, and magnesium was improved as well, while sodium and calcium uptake was not affected. Especially the increased uptake of nitrogen and phosphorous by plants and thus a more efficient use of fertilizers are very important in terms of nutrient legislation in high input cropping systems.

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