

# Effect of humic substances on nutrient uptake by herbage and on production and nutritive value of herbage from sown grass pastures

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## Abstract

The effect of humic substances on the nutrient uptake, herbage production and nutritive value of herbage from sown grass pastures was studied in six field experiments. Commercial humic substances were applied in combination with mineral fertilizer or slurry, either as a solution (HF liquid; 8.3 kg humic substances ha<sup>-1</sup>) or incorporated into the mineral fertilizer (HF incorporated; 3.6 to 6.4 kg humic substances ha<sup>-1</sup>). A series of cuts, ranging from two to five cuts, was taken during the growing season. The general response in herbage production to application of humic substances was an increase in herbage mass of dry matter (DM) at the first cut although this was only significant in two experiments for the HF incorporated treatment. Total herbage production of DM over the growing season, however, was similar for treatments with or without application of humic substances. The overall effect of HF incorporated and HF liquid on the herbage mass of DM at the first cut across the experiments was calculated using a meta-analysis technique and it was shown that there was a significant proportional increase of 0.14 ( $P < 0.05$ ) with the HF incorporated treatment and a non-significant increase of 0.08 with the HF liquid treatment compared to the control treatment. The nutritive value of the herbage at the first cut was similar across all treatments. In general nitrogen, phosphorus and potassium uptake at the first grass cut was higher after application of humic substances but only in one experiment was this increase statistically significant.

**Keywords:** humic substances, leonardite, grass, yield, nutrients, nutritive value

## Introduction

Many European agricultural regions with high inputs of inorganic fertilizers or organic fertilizer as slurry are faced with decreasing organic matter (OM) content of their soils (Vleeshouwers and Verhagen, 2002). In Belgium, the OM content of more than 0.50 of arable land and permanent grasslands is lower than the optimal value (Sleutel *et al.*, 2003; Boon *et al.*, 2009). As soil OM is essential for sorption and gradual release of nutrients, large amounts of external organic sources are needed to increase the OM content of the soil. However, application of high quantities of organic materials, which are usually rich in nitrogen (N) and phosphorus (P), is in conflict with legislation on nutrient inputs in many countries in north-west Europe.

The use of concentrated products of humic substances (humic and fulvic acids) in agriculture is one solution as many of the beneficial characteristics of soil humus are associated with the presence of humic and fulvic acids. These humic substances 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).

Many beneficial effects of humic substances on the growth of plants are recognized as they can produce various morphological, physiological and biochemical effects on plants (see reviews by Visser, 1986; Chen and Aviad, 1990 and Nardi *et al.*, 2002). Numerous studies have shown that the addition of a specific amount of humic substances can enhance the growth of roots, shoots and leaves, and encourage nutrient absorption by plants. Extended reviews were published by Varanini and Pinton (2001) and Nardi *et al.* (2002). The effects of humic substances on growth and metabolism

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of maize have been studied by many authors (Canellas *et al.*, 2002; Sharif *et al.*, 2002; Quagiotti *et al.*, 2004; Nardi *et al.*, 2007; Eyheraguibel *et al.*, 2008; Carletti *et al.*, 2008; Canellas *et al.*, 2008; Verlinden *et al.*, 2009). Other crops studied have been barley (Ayuso *et al.*, 1996), wheat (Delfine *et al.*, 2005; Jones *et al.*, 2007), potato (Verlinden *et al.*, 2009), olive plants (Fernandez-Escobar *et al.*, 1996), tomato (Atiyeh *et al.*, 2002; Yildirim, 2007), lettuce (Tufencki *et al.*, 2006) and strawberry (Pilanah and Kaplan, 2003; Arancon *et al.*, 2006). Comparison of the different studies showed that the effects of humic substances may be affected by the origin, concentration and molecular weight of the humic substances, and the species and variety of the plant treated (Visser, 1986; Chen and Aviad, 1990; Varanini and Pinton, 2001; Nardi *et al.*, 2002).

The effect of humic substances in grass pastures has rarely been reported. However, if OM content of grass pastures continues to decrease, grass pastures could also benefit from the use of humic substances. Cooper *et al.* (1998) showed that humic substances could increase root mass of creeping bentgrass (*Agrostis stolonifera* L.) proportionately by 0.45 in the 0–10 cm soil layer and increase P concentration of the herbage proportionately by 0.03–0.05. Hafidi *et al.* (1997) measured an increased P absorption by 0.19–0.35 in Italian ryegrass herbage (*Lolium multiflorum* L.) following application of different extracts of humic substances. The experiments of Cooper *et al.* (1998) and Hafidi *et al.* (1997) were set up in greenhouse conditions and did not assess the effect of humic substances on herbage production and its mineral composition.

The aim of this study was a simultaneous assessment of the effect of humic substances on nutrient uptake, herbage production and the nutritive value of the herbage. Field experiments were preferred to greenhouse experiments in order to interpret the effects of humic substances in the light of environmental conditions. General conclusions about the effect of humic substances on herbage production, nutrient uptake and nutritive value of the herbage between the different experiments were obtained by applying the meta-analysis technique of Hedges *et al.* (1999) and Gurevitch and Hedges (2001). This method allows the integration of results of independent field experiments.

## Materials and methods

### Humic substances

A liquid mixture of humic and fulvic acids (Humifirst®; Tradecorp, Madrid, Spain) was used as an organic amendment. The mixture, termed HF liquid, contained 0.12 of humic acids and 0.03 of fulvic acids (weight/

weight solution). The HF liquid was sprayed on the grass herbage at the start of the growing season. Besides the liquid form, the solid form, Humifirst WG, containing 0.68 of humic substances, was also used in the experiments. Humifirst WG was incorporated into mineral fertilizers at varying concentrations (Table 1) and will be subsequently referred to as HF incorporated. The humic substances in both products were extracted from Canadian Leonardite which is highly oxidized lignite with more than 0.85 of humic acids.

### Site and experimental designs

Field experiments with humic substances were carried out in 2006, 2007 and 2008 at four locations in Flanders, the northern part of Belgium. In 2006 Experiments BOT1 and BOT2 were set up at Bottelare (*L. multiflorum* pasture), Experiment HOOG was set up at Hoogstraten (*L. perenne* pasture) and Experiment MER1 was set up at Merelbeke (*L. perenne* pasture). In 2007 Experiment BOT2 was continued with the same treatments and Experiment HOOG was continued with some additional treatments (Table 1). In 2008, two new experiments were set up at Merelbeke (MER2, *L. perenne* pasture) and at Moortsele (MOOR, *L. perenne* pasture). Soil characteristics on each location are shown in Table 2.

The humic acid treatments in each experiment are shown in Table 1. In Experiments BOT1, BOT2, MER2 and MOOR, three treatments were imposed: (i) a control treatment with mineral fertilizer according to fertilizer recommendations based on soil chemical analyses, (ii) mineral fertilizer + 50 L ha<sup>-1</sup> HF liquid and (iii) mineral fertilizer with HF incorporated. In Experiment MER1 only two treatments were imposed: (i) a control treatment with mineral fertilizer according to fertilizer recommendations based on soil chemical analyses and (ii) mineral fertilizer with HF incorporated. In Experiment HOOG the same treatments as in MER1 were applied as well as three treatments with slurry application: (i) slurry application plus mineral fertilizer, (ii) slurry application mixed with 50 L ha<sup>-1</sup> HF liquid + mineral fertilizer and (iii) slurry application plus mineral fertilizer with HF incorporated. For all experiments, a randomized complete block design was used with four replicates. Plot size varied between 19 m<sup>2</sup> for Experiment MER1 and 102 m<sup>2</sup> for Experiment HOOG, with an average plot size of 45 m<sup>2</sup>. On all locations, except in Experiment BOT2, the experiments were set up on grasslands of more than 1 year old which were not included in a crop rotation. The exception was Experiment BOT2 where grass seed was sown in April 2006. Consequently, results of a full growing season could only be assessed in 2007 at this location.

**Table 1** Experiments and treatments, including amounts of humic substances (HS) in the form of Humifirst (HF) as a liquid or incorporated into mineral fertilizer. Amount of mineral fertilizer based on chemical soil analysis and recommendations by the Soil Service of Belgium. Amounts of mineral fertilizer in each experiment are given as the amounts at the beginning of the season or after each cut in the sequence below and the values are expressed in kg ha<sup>-1</sup>: Experiment BOT1, N = 100/80, P<sub>2</sub>O<sub>5</sub> = 80/0, K<sub>2</sub>O = 100/100; Experiment BOT2 in 2006, N = 90/80/80, P<sub>2</sub>O<sub>5</sub> = 80/0/0, K<sub>2</sub>O = 130/80/0; Experiment BOT2 in 2007: N = 90/80/80, P<sub>2</sub>O<sub>5</sub> = 60/0/0, K<sub>2</sub>O = 130/80/80; Experiment HOOG in 2006 and 2007: N = 100/60/50/40, P<sub>2</sub>O<sub>5</sub> = 0/0/30/30, K<sub>2</sub>O = 170/100/100; Experiment MER1, N = 120/100/100/80, P<sub>2</sub>O<sub>5</sub> = 56/60/30/24, K<sub>2</sub>O = 120/100/100/80; Experiment MER2, N = 100/80/70, P<sub>2</sub>O<sub>5</sub> = 70/20/0, K<sub>2</sub>O = 120/100/80; Experiment MOOR, N = 90/70/50, P<sub>2</sub>O<sub>5</sub> = 70/0/20, K<sub>2</sub>O = 90/80/70.

Experiment	Treatments		Amount of HS (kg ha <sup>-1</sup> )	Amount of HS (kg ha <sup>-1</sup> )
	2006	2007		
BOT1	1. Mineral fertilizer		0	
	2. Mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid		8.3	
	3. Mineral fertilizer + 0.015 HF incorporated		6.4	
BOT2	1. Mineral fertilizer	1. Mineral fertilizer	0	0
	2. Mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid	2. Mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid	8.3	8.3
	3. Mineral fertilizer + 0.015 HF incorporated	3. Mineral fertilizer + 0.015 HF incorporated	3.6	3.6
HOOG	1. Mineral fertilizer	1. Mineral fertilizer	0	0
	2. Mineral fertilizer + 0.035 HF incorporated	2a. Mineral fertilizer + 0.035 HF incorporated in 2006	5.7	0
		2b. Mineral fertilizer + 0.035 HF incorporated		5.7
		in 2006 and 2007		
	3. Slurry application (20 tons ha <sup>-1</sup> ) + mineral fertilizer	3. Slurry application + mineral fertilizer	0	0
4. Slurry application + mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid	4. Slurry application + mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid	8.3	8.3	
5. Slurry application + mineral fertilizer + 0.035 HF incorporated	5a. Slurry application + mineral fertilizer + 0.035 HF incorporated in 2006	5.7	0	
	5b. Slurry application + mineral fertilizer + 0.035 HF incorporated in 2006 and 2007		5.7	
MER1	1. Mineral fertilizer		0	
	2. Mineral fertilizer + 0.035 HF incorporated		8.3	
		2008	Amount of HS (kg ha <sup>-1</sup> )	
MER2	1. Mineral fertilizer		0	
	2. Mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid		8.3	
	3. Mineral fertilizer + 0.015 HF incorporated		6.4	
MOOR	1. Mineral fertilizer		0	
	2. Mineral fertilizer + 50 L ha <sup>-1</sup> HF liquid		8.3	
	3. Mineral fertilizer + 0.035 HF incorporated		6.5	

**Table 2** Characterization of the six experimental sites. The soil chemical parameters were determined from samples taken in the 0–6 cm layer of the soil except in Experiment BOT2 where samples were taken in the 0–30 cm soil layer.

Soil variable	Experiment											
	BOT1		BOT2		HOOG		MER1		MER2		MOOR	
Soil type	Loamy sand*		Loamy sand		Sand		Sand		Sandy loam		Sandy loam	
Location	Bottelare		Bottelare		Hoogstraten		Merelbeke		Merelbeke		Moortsele	
OM content (% C)	1.1	Very low†	0.8	Very low	1.8	Very low	1.6	Very low	1.3	Very low	4.8	Fairly high
pH-KCl‡	5.4	Optimal	5.2	Optimal	4.9	Fairly low	6.4	High	5.1	Fairly low	6	Fairly high
P content (mg 100 g <sup>-1</sup> )§	23	Fairly high	13	Low	44	High	16	Optimal	15	Fairly low	18	Fairly low
K content (mg 100 g <sup>-1</sup> )§	8	Fairly low	8	Low	14	Optimal	22	High	9	Fairly low	35	High
Mg content (mg 100 g <sup>-1</sup> )§	14	Optimal	6	Low	9	Low	11	Optimal	6	Low	55	Fairly high
Ca content (mg 100 g <sup>-1</sup> )§	59	Low	67	Low	88	Optimal	71	Optimal	62	Low	265	Optimal
Na content (mg 100 g <sup>-1</sup> )§	1.2	Low	1.3	Low	2.4	Low	2.5	Low	1.6	Low	3.8	Fairly low

\*According to the USDA-triangular diagram of soil textural classes.

†Classification of the respective soil parameter as a function of soil use, soil type and soil organic matter content used by the National Soil Service of Belgium (Boon *et al.*, 2009). Seven classes are distinguished: very low, low, fairly low, optimal, high, fairly high, very high.

‡pH-KCl (1M) determined in a soil-solution ratio of 1:1 (1 M potassium chloride).

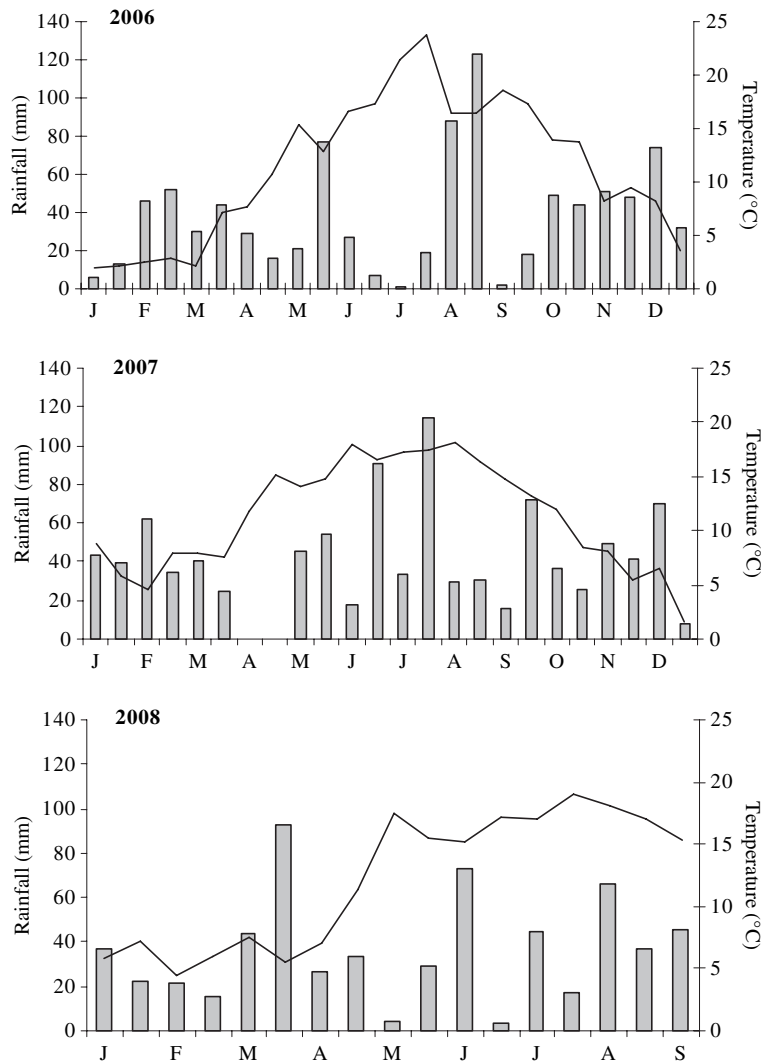
§Measured in ammonia-lactate extract.

The number of grass cuts at each location depended on the weather and grass conditions on each field and varied between two and five cuts per growing season. The aim was to have a herbage production between 2.5 and 4.5 tons DM ha<sup>-1</sup> for each cut. Only two cuts were taken in experiment BOT1 in 2006 (9 May and 22 June) due to the very dry period in July 2006 (Figure 1) and the subsequent degradation of *L. multiflorum*. In BOT2 four cuts were taken in 2006 and 2007, respectively on 22 June, 25 July, 5 September and 17 October in 2006 and 28 March, 26 April, 5 June and 9 July in 2007. The first cut in 2007 was performed just after winter before fertilizer and HF application as grass growth on this field was substantial during the mild winter period of 2006–2007. This situation provided a good opportunity to measure residual effects of humic substances. In experiment HOOG four cuts were taken in 2006 (8 May, 6 June, 13 July and 5 September) and in 2007 (20 April, 22 May, 30 July and 12 September). Both in experiment MER1 and MER2 five cuts were performed (MER1, 2006: 11 May, 21 June, 8 August, 8 September and 26 October; MER2, 2008: 13 May, 16 June, 22 July, 3 September and 28 October) while only three cuts were carried out in experiment MOOR in 2008 (14 May, 24 June and 17 November).

Fresh herbage mass at each cut was determined by collecting and weighing a subplot of at least 10 m<sup>2</sup>

(cutting height of approximately 5 cm). For each plot, a subsample of the freshly harvested herbage mass was weighed, dried at 60°C for 72 h and re-weighed to determine the dry matter (DM) content and to calculate the herbage mass of DM of each plot at each date. Herbage mass of DM of the different cuts were counted up to calculate total herbage production during the growing season.

The mineral composition and nutritive value of the herbage harvested was measured using subsamples collected to determine herbage mass on all cutting dates. Mineral composition was measured on all cutting dates and nutritive value was only measured at the first cut. The subsamples were ground using a sample mill (Retsch) to pass through a 0.5-mm sieve. Macro-nutrients (P, K, Mg, Ca and Na) concentrations in herbage were determined by ICP-AES after dry-ashing of the samples. Nitrogen was determined using a Vario Max analyzer after combustion of the samples in an oxygen-rich atmosphere at high temperatures (Dumas method). Crude protein and crude fibre concentrations and digestibility of OM were determined using near-infrared reflectance spectroscopy (Infrared Spectrometer XDS; FOSS-Benelux, Amersfoort, The Netherlands). The NIR spectrum in this method was calibrated with results obtained with reference methods for each parameter (Kjeldahl-method for crude protein content



**Figure 1** Mean daily temperature (solid line) and rainfall (bars) for 2006, 2007 and 2008 at location Bottelare. Each month is divided in period 1 'day 1 to 15' and period 2 'day 16 to end of the month'. The data for location Bottelare are representative of the other locations.

with a nitrogen to protein conversion factor of 6.25, Scharrer and Kurschner method (1931) for crude fibre content and De Boever *et al.* method (1986) for digestibility).

### Data analysis

In all data analyses, a distinction was made between the effects of HF liquid and HF incorporated. First, the change in herbage production, nutrient uptake and nutritive value due to the application of HF was calculated for each experiment as a proportion of the value of the control treatment (Tables 3, 5 and 6). Analysis of variance was used to study the effect of treatment (HF application) on DM production, nutrient uptake and nutritive value for each individual exper-

iment (Version 4.1, SAS package; SAS Institute Inc., Cary, NC, USA). A two-sample *t*-test was used to test if the average of the differences between the observations (with and without HF application) was significantly different.

To compare the results between experiments, a meta-analysis was performed in accordance with Hedges *et al.* (1999) and Gurevitch and Hedges (2001) using the log response ratio or the logarithm of the ratio of some measured quantity in an experimental and control group, as an index of effect. In this case, it is desirable to perform the statistical analysis in the metric of the natural logarithm of the response ratio as the distribution of the logged response ratio is much more normal in small samples than that of the unlogged ratio. In this meta-analysis the treatments with mineral fertilizer

**Table 3** Mean herbage mass of dry matter (DM) with standard deviation of mean in parentheses for the Control treatment of the cuts on the experimental sites. Humic substances in the form of Humifirst as a liquid (HF liq) or incorporated into mineral fertilizer (HF inc) were applied as treatments (see Table 1). The herbage mass of DM for these treatments are expressed as a ratio of the herbage mass of DM of the Control treatment.

Experiment Treatment	Year											
	2006				2007				2008			
	HOOG		HOOG		HOOG		HOOG		HOOG		HOOG	
	BOT1	BOT2	No slurry	Slurry	MER1	BOT2	No slurry	Slurry	MER2	MOOR		
Cut 1												
Control (kg ha <sup>-1</sup> DM)	3416 (741)	3668 (264)	2529 (208)	2166 (77)	4140 (304)	1781 (458)	2448 (704)	2436 (178)	3139 (61)	2367 (328)		
HF liq	1.12	0.96	–	1.18	–	1.16	–	–	1.06	1.11		
HF inc	1.03	0.85	1.42**	1.66**	1.09	1.03	1.08†	0.96†	1.11	1.05		
Cut 2												
Control (kg ha <sup>-1</sup> DM)	5711 (537)	2089 (225)	2175 (369)	1977 (243)	3814 (231)	3584 (90)	1886 (93)	1825 (142)	2943 (143)	4353 (341)		
HF liq	0.94	1.04	–	0.92	–	1.01	–	–	1.13**	0.92		
HF inc	0.89	0.91	0.87	0.90	1.03	0.98	1.07	1.10*	0.99	1.02		
Cut 3												
Control (kg ha <sup>-1</sup> DM)		2785 (58)	1701 (491)	1520 (72)	819 (36)	3944 (426)	2751 (1172)	3195 (1365)	4331 (306)	2813 (453)		
HF liq		1.07	–	0.85	–	0.91	–	–	1.17	0.88		
HF inc		1.07	0.86	1.03	1.18*	0.97	1.14	1.08	1.02	1.05		
Cut 4												
Control (kg ha <sup>-1</sup> DM)		1347 (228)	1707 (146)	1940 (217)	2082 (26)	1549 (430)	1578 (142)	1731 (125)	2432 (187)			
HF liq		0.95	–	1.00	–	0.69	–	–	0.87			
HF inc		0.87	1.14	1.00	1.05	0.75	0.91	0.77*	0.93			
Cut 5												
Control (kg ha <sup>-1</sup> DM)					1401 (22)				1210 (190)			
HF liq					–				0.95			
HF inc					0.79**				0.80			
Total herbage production												
Control (kg ha <sup>-1</sup> DM)	9127 (1163)	9889 (475)	8113 (796)	7603 (448)	12 256 (167)	10 857 (1220)	8663 (1524)	9187 (1390)	14 056 (447)	9534 (490)		
HF liq	1.01	1.01	–	1.00	–	0.95	–	–	1.07	0.96		
HF inc	0.94	0.93	1.10	1.17**	1.04	0.95	1.06†	1.04†	1.00	1.04		

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$  are significant differences between HF and control treatment.

†HF applied in 2006.

‡HF applied in 2006 and 2007.

without humic substances were considered as the control group (Xc) and the treatments with application of humic substances as the experimental group (Xe). The meta-analysis was performed separately for HF liquid (class 1) and HF incorporated (class 2) and was carried out using the experimental data of the total growing season and the data of the first cut as this cut is the most important in grassland exploitation through its high nutritive value. Table 4 shows the selection of the experiments which were included in the meta-analysis. As data within each class have to be independent, only one experimental year per experiment could be included in the meta-analysis. The first full growing season was chosen per experiment.

Both herbage production and nutrient uptake were considered as an index of effect of humic substances. In the following paragraphs the calculations for the index 'herbage production' will be described in detail. The first step in the meta-analysis was the calculation of the log response ratio  $L_i$  as the natural logarithm of the mean herbage production of the experimental group ( $\bar{X}_e$ ), or herbage production with application of humic substances, to the mean herbage production of the control group ( $\bar{X}_c$ ) or the herbage production without application of humic substances. The 100 (1 -  $\alpha$ ) percentage confidence interval for the individual log response ratio parameter  $\lambda$  is given by  $L - z_{\alpha/2}\sqrt{v} \leq \lambda \leq L + z_{\alpha/2}\sqrt{v}$  with  $z_{\alpha/2}$  the 100(1 -  $\alpha/2$ ) percentage point of the standard normal distribution and variance  $v_i = \frac{(SD_e)^2}{n_e X_c^2} + \frac{(SD_c)^2}{n_c X_c^2}$  (where  $n$  is sample size and SD is standard deviation of mean). The corresponding unlogged response ratio and its confidence interval is obtained by taking the antilogs of the log response ratio and of the confidence limits for the log response ratio. The following step in the meta-analysis is the calculation of the cumulated mean effect size of humic substances on herbage production across the different

**Table 4** The experiments contained in the two classes of the meta-analysis of the experiments, with class 1 containing the comparisons between the Control treatment and the HF liquid treatment and class 2 containing the comparisons between the Control treatment and the HF incorporated treatment. See Table 1 for description of treatments. Data within each class have to be independent and, therefore, only one experimental year per location can be included in the meta-analysis. The first full growing season was chosen per location.

Class 1 – HF liquid	Class 2 – HF incorporated
Experiment BOT1 in 2006	Experiment BOT1 in 2006
Experiment BOT2 in 2007	Experiment BOT2 in 2007
Experiment MER2 in 2008	Experiment HOOG in 2006
Experiment MOOR in 2008	Experiment MER1 in 2006
	Experiment MER2 in 2008
	Experiment MOOR in 2008

experiments and the use of a random effect model (Gurevitch and Hedges, 2001). The cumulated mean effect size or mean log response ratio  $\bar{L}^*$  is a weighted mean of the log response ratios of the different experiments or

$$\bar{L}^* = \frac{\sum_{i=1}^k w_i^* * L_i}{\sum_{i=1}^k w_i^*},$$

where  $k$  is number of experiments and  $w_i^* = \frac{1}{(v_i + \sigma_i^2)}$ , the reciprocal of the total variance of  $L_i$ . The total variance of  $L_i$  consists of two components of variation, the sampling variation for each experiment,  $v_i$ , and the between-experiment variation,  $\sigma_i$ . The combination of experimental data is rejected if the between-experiment variation is too large compared to the average within-experiment sampling-error variation. This was the case for the nutrient uptake data in this meta-analysis and thus calculations were only undertaken for the herbage production data. The 100(1 -  $\alpha$ ) percentage confidence interval for the mean log response ratio,  $\mu_\lambda$ , is given by  $\bar{L}^* - z_{\alpha/2}SE(\bar{L}^*) \leq \mu_\lambda \leq \bar{L}^* + z_{\alpha/2}SE(\bar{L}^*)$  where

$$SE(\bar{L}^*) = \sqrt{\left(\frac{1}{\sum_{i=1}^k w_i^*}\right) \left(1 + 4 \sum_{i=1}^k \frac{1}{df_i} \left(\frac{w_i^*}{w_i}\right)^2 \frac{w_i^* \left(\left(\sum_{i=1}^k w_i^*\right) - w_i^*\right)}{\left(\sum_{i=1}^k w_i^*\right)}\right)}$$

and  $df_i$  is the number of degrees of freedom in the  $i$ th experiment ( $n_e + n_c - 2$ ).

## Results

### Effects of humic substances on herbage production

For the first cut in Experiments BOT1, MER1, MER2, HOOG and MOOR, application of humic substances in combination with mineral fertilizer resulted in a positive response in herbage mass of DM on all pastures but few of the responses were statistically significant. In Experiment HOOG (2006) the proportional increase in herbage mass of DM at the first cut of 0.42 for HF incorporated compared to control was highly statistical significant and in Experiment MER2, HF incorporated increased the herbage mass of DM significantly by 0.11. The application of HF liquid in combination with mineral fertilizer did not increase herbage mass of DM significantly (Table 3).

In the meta-analysis of the data on the first cut, a summary of the log response ratios calculated for the individual experiments and the mean log response ratio calculated across the experiments is shown in Figure 2a,c. Application of HF incorporated resulted in

**Table 5** Nutrient uptake (N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) at the first cut and total uptake during growing season with standard deviation of mean in parentheses for the Control treatment of the cuts on the experimental sites. Humic substances in the form of Humifirst as a liquid (HF liq) or incorporated with mineral fertilizer (HF inc) were applied as treatments (see Table 1). The nutrient uptakes for these treatments are expressed as a ratio of the nutrient uptakes of the Control treatment.

Year	Experiment	Cut	Slurry application	Humic acids application						N uptake			P <sub>2</sub> O <sub>5</sub> uptake			K <sub>2</sub> O uptake		
				2006	2007	2008	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc
				2006	2007	2008	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc	Control (kg ha <sup>-1</sup> )	HF liq	HF inc
2006	BOT1	Cut 1	No	x		80.4 (13.0)	1.08	1.08	26.0 (12.6)	1.01	1.11	81.0 (4.9)	1.10	1.11				
		Total			187.1 (15.5)	1.05	1.04	59.7 (28.7)	0.95	1.00	213.0 (8.3)	0.96	0.98					
	BOT2	Cut 1	No	x		100.0 (10.5)	1.01	0.93	24.8 (3.7)	0.96	0.88	153.0 (7.6)	1.00	0.93				
		Total*			267.1 (12.8)	1.01	0.92*	55.4 (7.5)	1.03	0.96	375.3 (11.6)	1.01	0.98					
HOOG	Cut 1	No	x		57.1 (4.8)	-	1.72***	19.7 (1.7)	-	1.84***	66.4 (10.2)	-	1.97**					
		Yes	x		53.2 (6.0)	1.40	1.84**	19.9 (1.0)	1.30	1.83***	71.9 (5.6)	1.28	1.81***					
	Total	No	x		194.6 (22.6)	-	1.19*	76.6 (7.2)	-	1.16*	299.1 (37.7)	-	1.16					
		Yes	x		191.5 (3.3)	1.01	1.20***	74.9 (1.6)	1.00	1.22***	292.9 (3.1)	0.97	1.22*					
2007	BOT2	Cut 1	No	x	x	26.1 (5.7)	1.15	1.05	8.4 (2.0)	1.25	1.11	33.8 (5.5)	1.29	1.14				
		Cut 1	No	x	x	74.9 (15.0)	-	1.06	-	-	1.12	-	-	-	-	-		
	HOOG	No	x	-														
		Yes	x	x		77.9 (4.7)	1.03	0.93										
MOOR	Cut 1	No	x		233.5 (36.8)	-	1.02											
		Yes	x		251.3 (37.3)	1.04	0.92											
	Total†	No	x		53.7 (6.0)	1.08	1.01	19.1 (2.5)	1.10	1.05	82.3 (13.3)	1.12	1.10					
		Yes	x		205.7 (-)	0.95	1.07	75.5 (-)	0.93	1.01	302.7 (-)	0.97	1.06					
MER2	Cut 1	No	-		78.4 (3.2)	1.05	1.10	33.1 (1.4)	1.07	1.10	125.8 (7.1)	0.97	1.11					
	Total‡	No	-		316.0 (-)	1.09	1.06	120.0 (-)	1.08	1.03	493.0 (-)	1.03	1.08					

\*Total of 3 cuts, no data available for cut 4.

†Only at cut 1, analysis was performed for each grass sample replication. At cut 2 to cut 4, analysis was performed for a mixed grass sample made of the 4 replications per treatment.

‡Total of 4 cuts, no data available for cut 5.

\*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001 are significant differences between HF and control treatment.

**Table 6** Crude protein (CP) and crude fibre (CF) concentrations and digestibility of organic matter (OM) of herbage from the first cut in Experiments BOT1, BOT2, HOOG, MOOR and MER2. Values in parentheses are standard deviation of mean. Humic substances in the form of Humifirst as a liquid (HF liq) or incorporated with mineral fertilizer (HF inc) were applied as treatments (see Table 1). The nutritive variables for these treatments are expressed as a ratio of the nutritive variables of the Control treatment.

Year	Experiment	Slurry application	Humic acids application				CP concentration				CF concentration				Digestibility of OM			
			2006	2007	2008		Control	HF liq	HF inc		Control	HF liq	HF inc		Control	HF liq	HF inc	
						(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)	(g kg <sup>-1</sup> DM)
2006	BOT1	No	x			165 (9.0)	0.94	-		236 (6.0)	0.97	-		0.834 (0.004)	1.00	-		
	BOT2	No	x			188 (11.0)	1.03	1.01		222 (2.0)	1.01	0.99		0.907 (0.024)	1.00	1.00		
2007	HOOG	No	x	x		195 (21.0)	-	0.99		176 (8.0)	-	1.02		0.892 (0.018)	-	1.03		
		No	x	-		195 (21.0)	-	1.02		176 (8.0)	-	1.03		0.892 (0.018)	-	1.03		
		Yes	x	x		200 (11.0)	0.99	0.97		175 (5.0)	1.01	1.00		0.907 (0.025)	1.00	0.99		
		Yes	x	-		200 (11.0)	-	0.99		175 (5.0)	-	1.03		0.907 (0.025)	-	0.99		
2008	MOOR	No	-	-	x	156 (10.0)	0.98	0.97		230 (2.0)	1.03	1.04*		0.827 (0.007)	0.99	0.99		
	MER2	No	-	-	x	163 (6.0)	0.98	1.00		283 (5.0)	0.98	0.99		0.780 (0.009)	1.00	0.99		

\*,  $P < 0.05$  is significant difference between HF and control treatment.

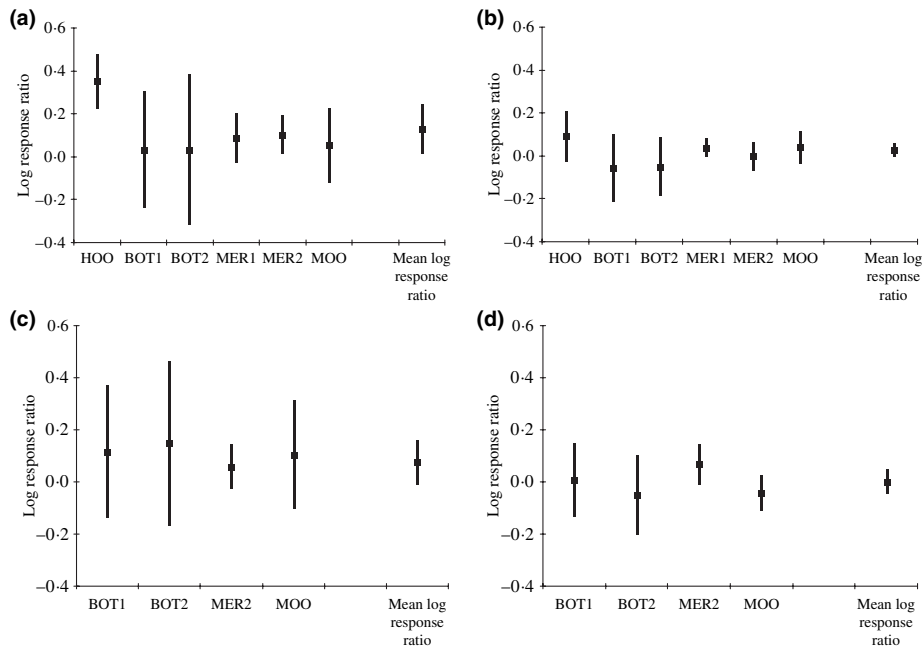
a mean log response ratio of 0.13 or an unlogged mean response ratio of 1.14 with the unlogged 95 % confidence interval of 1.02–1.27 for the first cut. In other words, the application of HF incorporated significantly ( $\alpha = 0.05$ ) increased grass production by 0.14 at the first cut across the different field experiments. The application of HF liquid resulted in a mean log response ratio of 0.07 or an unlogged mean response ratio of 1.08. The increase in grass production of 0.08 compared to the control was not significant. Higher herbage masses of DM at the first cut after application of humic substances in combination with mineral fertilization were often followed by lower herbage masses of DM in the following cuts (Table 3).

The effect of humic substances in combination with mineral fertilizer on total herbage production of DM at the end of the growing season was variable. Mean total herbage production of DM on plots with humic substances and mineral fertilizer varied from -0.07 to +0.10 compared to the means on the control plots (Table 3). In the meta-analysis the mean log response ratio across the experiments was estimated to be zero for HF liquid and 0.03 for HF incorporated. This means that in general the application of humic substances had no effect on total herbage production of DM during the growing season.

In Experiment HOOG, humic substances were applied in combination with mineral fertilizers and slurry as well. The general trend observed by application of humic substances, as described above, persisted. Incorporation of HF into the mineral fertilizer resulted in a significant increase of 0.66 in herbage mass of DM at the first cut. Total herbage production of DM increased significantly by 0.17 compared to the control treatment. When HF liquid was mixed into the slurry, herbage mass of DM at the first cut increased by 0.18 but total herbage production of DM at the end of the growing season was comparable to the control treatment.

### Effect of humic substances on nutrient uptake by herbage

The uptake of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O by the herbage in control plots (in kg ha<sup>-1</sup>) and the effect of the application of humic substances on the proportional change in uptake compared to the control is presented in Table 5. At the first cut, N uptake always increased after application of humic substances in combination with mineral fertilizer. However, this effect could only be statistically demonstrated in Experiment HOOG in 2006 for the HF incorporated treatment. Total N uptake during the growing season generally increased with application of humic substances but, again, the increased nitrogen uptake was only statistically



**Figure 2** Log response ratios of (a) HF incorporated and (c) HF liquid for the herbage mass of dry matter (DM) at the first cut and (b) HF incorporated and (d) HF liquid for the total herbage production of DM over the growing season as calculated in the meta-analysis. In each graph, log response ratios of the individual experiments are shown on the left side and the mean response ratio across the different experiments is shown on the right side. Error bars indicate 95% confidence intervals.

significant in Experiment HOOG in 2006 for the HF incorporated treatment.

The uptake of  $K_2O$  and  $P_2O_5$  was similar at the different locations. The application of humic substances resulted in a positive effect on  $K_2O$  and  $P_2O_5$  uptake at the first cut and total  $K_2O$  and  $P_2O_5$  uptake in Experiment HOOG for the HF incorporated treatment. The effect of humic substances on  $MgO$ ,  $CaO$  and  $Na_2O$  uptake was similar to that for the other macro-nutrients in that uptake  $MgO$ ,  $CaO$  and  $Na_2O$  at the first cut and the total uptake of  $MgO$ ,  $CaO$  and  $Na_2O$  was higher in Experiment HOOG for the HF incorporated treatment (data not shown). Since the between-experiment variation for nutrient uptake data was too large compared to the average within-experiment variation in sampling error, the combination of experimental data was rejected by the meta-analysis.

### The effect of humic substances on nutritive value of herbage

Table 6 shows the results of crude protein and crude fibre concentrations and digestibility of the spring grass in the different experiments. If analysed, the crude protein and the crude fibre content of the spring grass varied from 156 to 195 g  $kg^{-1}$  DM and from 176 to

283 g  $kg^{-1}$  DM at the different cutting dates. The digestibility of DM of the herbage ranged from 0.78 to 0.90. The effect of humic substances on these values at the first cut was very small.

### Discussion

The results in the field experiments showed that application of HF incorporated increased grass production at the first cut without affecting the nutritive value of the herbage although there was no effect on annual herbage production. The shift in total herbage production towards the first cut during the growing season after application of humic substances, especially HF incorporated, could be of significance for grassland management in north-west Europe since the first cut is important as a source of nutrients for the winter as silage.

The higher grass production at the first cut was generally followed by lower production in the next cuts. This decline in herbage production after a high-yielding first cut is a well known phenomenon (Behaeghe, 1979). It is argued that the herbage uses a high proportion of its nutrient reserves in the roots for the production of spring grass so that regrowth is probably hampered by a lack of reserves.

Significant increases in N uptake by herbage at the first cut associated with the addition of humic substances could only be demonstrated in one of the experiments for the HF incorporated treatment and it was not possible to undertake the meta-data analysis because of the high variability. Increases in yield and nutrient uptake following application of humic substances in combination with mineral fertilizer have been reported for barley (Burns *et al.*, 1986; Ayuso *et al.*, 1996), olive trees (Fernandez-Escobar *et al.*, 1996) and maize (Pinton *et al.*, 1999). Cooper *et al.* (1998) and Sharif *et al.* (2002) found that the effect of humic substances increased if they were mixed into the nutrient solution. This supports the larger effect of HF incorporated compared to HF liquid since nutrients and humic substances are in the same granule in the 'HF incorporated' product.

The reasons for the increase in herbage mass of DM and N uptake at the first cut with the HF incorporated treatment were not studied. In studies with other crop species, an increase in N uptake may be explained by the role of humic substances in the modulation of nitrate uptake via an interaction with plasma membrane H<sup>+</sup>-ATPase (Maggioni *et al.*, 1987; Pinton *et al.*, 1999; Canellas *et al.*, 2002; Quagiotti *et al.*, 2004).

In the study of Pinton *et al.* (1999) the presence of nitrate and humic substances resulted in a similar stimulation of the nitrate uptake capacity and the plasma membrane H<sup>+</sup>-ATPase activity. Quagiotti *et al.* (2004) showed that humic substances exert direct effects on gene transcription in roots (genes encoding maize H<sup>+</sup>-ATPase and thus promoting nitrate influx in roots) and long-distance effects in shoots (effect on nitrate transporters and thus promoting nitrate accumulation in leaves). These effects are considered as an important action of humic substances on plant nutrient acquisition. Another important factor in the uptake of nutrients is the root system of plants. Cooper *et al.* (1998) found that incorporation of humic substances in the soil stimulated root mass of creeping bentgrass by 0.45 in the 0–10 cm soil layer and by 0.38 in the 10–20 cm soil layer. A more intensive root system is also essential for water uptake by plants as larger soil volumes can be explored for water. Moreover, plants treated with humic substances have a higher water efficiency than non-treated plants which means that the plants are able to produce more biomass for the same consumption of water (Eyheraguibel *et al.*, 2008). It would be interesting to examine the latter in further research if weather conditions become more extreme in the future.

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