

Article

The effect of an engineered biostimulant derived from *Ascomyces nodosum* on grass yield under a reduced nitrogen regime in an agronomic setting

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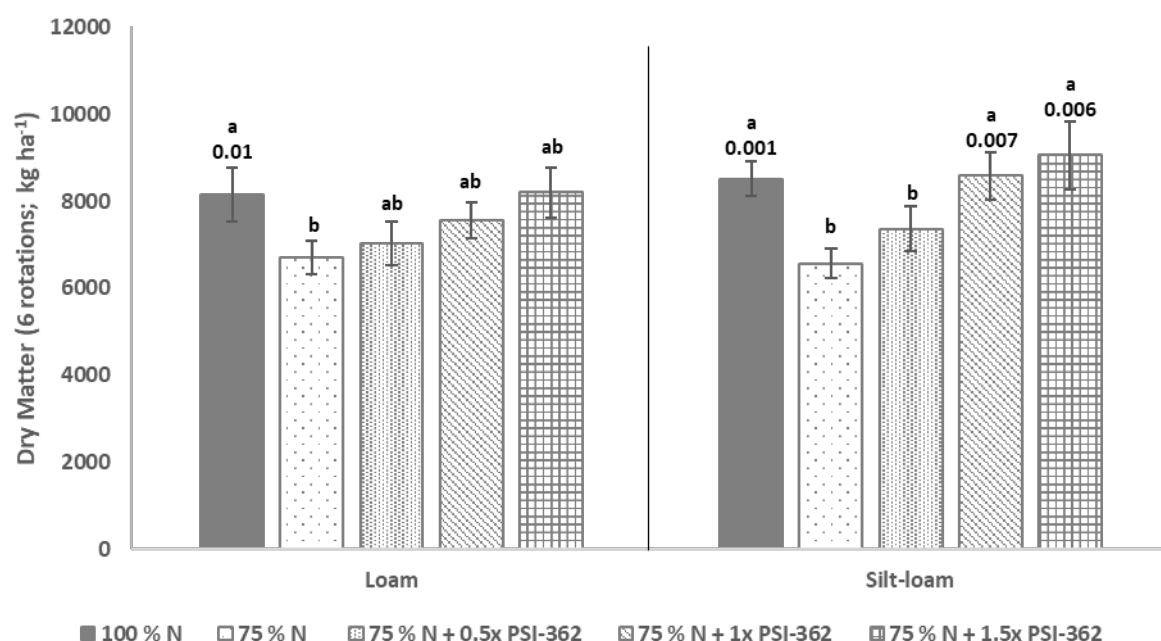
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Supplementary Figure S1: Modified lysimeter set-up



Supplementary Figure S2: Performance of PSI-362 coated fertilizer sorted according to soil type. Note: figures from the loam soils are the average of 2 soils (n = 6 for each soil; n = 12) over two years of six rotations, while figures for silt loam soils are the average of 4 soils (n = 6 for each soil; n = 24) over two years of six rotations. Values present means ± SE. Significance is indicated by different letters while P values represent significance levels compared to the 75 % N control (Kruskal-Wallis).

Supplementary Table S1: Soil attributes from the six different soils used for the modified lysimeter trials.

Soil no.	Sand %	Silt %	Clay %	Soil Texture	pH	P	K
				Class		(mg/L; index*)	(mg/L; index*)
Soil 1	49	40	11	Loam	6.9	42.9; 4	63.0; 2
Soil 2	26	50	24	Loam	6.7	6.8; 3	88.8; 2
Soil 3	8	71	21	Silt Loam	5.6	6.9; 3	51.2; 2
Soil 4	25	62	13	Silt Loam	7.1	38.6; 4	81.2; 2
Soil 5	24	60	16	Silt Loam	6.4	11.4; 4	116.0; 3
Soil 6	33	53	14	Silt Loam	7.0	30.6; 4	91.9; 2

*Indexes range from 1 to 4 as described by Wall and Plunkett

Supplementary Table S2: The fertilizer regime applied to the modified lysimeter trials according to recommended rates by Wall and Plunkett

Programme	Nutrient	Fertiliser	Month of Application								
		Rate	Feb	Mar	Apr	May	June	July	Aug	Sep	
100 % N	N applied (kg ha ⁻¹)	100 %	31	54	54	56	24	24	24	37	
	P applied (kg ha ⁻¹)	100 %	10.5	-	-	-	-	-	-	-	
	K applied (kg ha ⁻¹)	100 %	21	-	-	-	-	-	-	-	
75 % N	N applied (kg ha ⁻¹)	75 %	23	40.5	40.5	42	18	18	18	28	
	P applied (kg ha ⁻¹)	100 %	10.5	-	-	-	-	-	-	-	
	K applied (kg ha ⁻¹)	100 %	21	-	-	-	-	-	-	-	

Supplementary Table S3: Fertilizer regime, soil fertility (P and K) index and coordinates of the 2020 and 2021 field trials

2020 Field Trials						
	Farm 1		Farm 2	Farm 3	Farm 4	Farm 5
P and K index	3 & 4		3 & 3	2 & 3	4 & 3	4 & 3
100 % N Added (kg ha ⁻¹)	66.5		66.5	66.5	66.5	66.5
80 % N Added (kg ha ⁻¹)	53.0		53.0	53.0	53.0	53.0
Coordinates	53°22'26.2"N		53°15'34.5"N	53°15'16.1"N	53°17'08.9"N	53°17'23.8"N
	7°43'39.3"W		7°32'53.2"W	7°34'46.4"W	8°52'50.1"W	8°52'19.0"W
Days Growth	46		39	46	43	43
2021 Field Trials						
	1 st cut silage		2 nd cut silage			3 rd cut silage
	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	Farm 11
P and K index	2 & 3	3 & 3	3 & 2	3 & 2	3 & 3	3 & 2
P and K added (kg ha ⁻¹)	C: 10.7 & 42.7	C: 11 & 45 T: 9 & 54	C: 8.3 & 33.7	C: 8.0 & 36.6	C: 9.5 & 42.7	C: 8.1 & 36.5
	T: 8.5 & 51.2		T: 6.7& 40.2	T: 6.1 & 36.6	T: 7.3 & 43.9	T: 6.1 & 36.5
100 % N Added (kg ha ⁻¹)	102.4	112.6	80.5	76.8	89.8	76.5
Reduced N Added	85.4 (17 % less N)	94.0 (20 % less N)	67.1 (17 % less N)	61.7 (21 % less N)	73.2 (18 % less N)	60.8 (21 % less N)
Coordinates	53°23'58.1"N		53°17'50.2"N	53°24'29.5"N	53°19'29.5"N	53°32'47.9"N
	7°34'27.9"W		7°35'36.9"W	7°36'05.1"W	7°60'76.9"W	7°43'49.7"W
Days Growth	54		44	45	44	45
			46			

C = 100 % N control; T = PSI-362 treated N (at reduced N rate)

Supplementary Table S4: Weather Data for lysimeter and field trials.

	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Rainfall (sum) and soil T (average)
Lysimeters										
2020 Rainfall (mm)	-	-	-	18.6	67	121.4	134.4	64.4	185.2	591.0
2020 10 cm Soil T (°C)	-	-	-	12.5	14.5	16.7	17.3	15	11	14.5
2021 Rainfall (mm)	181.6	70.6	23.8	135.6	26	97.8	90.6	-	-	626.0
2021 Mean 10 cm Soil T (°C)	6.5	7.9	9.7	11.5	15.4	17.8	16.4	-	-	12.2
Silage Harvesting Trials										
Farms 1, 2, 3 (2020 data)										
Rainfall (mm)	-	-	-	-	-	126.3	114	-	-	240.3
LTA (mm)	-	-	-	-	-	71.1	86.1	-	-	157.2
Mean 10 cm Soil T (°C)	-	-	-	-	-	15.5	15.8	-	-	15.7
Farm 4, 5 (2020 data)										
Rainfall (mm)	-	-	-	-	-	116.3	113.5	-	-	229.8
LTA (mm)	-	-	-	-	-	86.5	107.8	-	-	194.3
Mean 10 cm Soil T (°C)	-	-	-	-	-	15.1	16.0	-	-	15.6
Farm 6, 7, 8 (2021 data)										
Rainfall (mm)	-	-	25.5	107.4	-	-	-	-	-	132.9
LTA (mm)	-	-	65.9	69.2	-	-	-	-	-	135.1
Mean 10 cm Soil T (°C)	-	-	7.8	10.5	-	-	-	-	-	9.2
Farm 10 (2021 data)										
Rainfall (mm)	-	-	-	-	25.7	57.8	-	-	-	83.5
LTA (mm)	-	-	-	-	74.2	73.1	-	-	-	147.3
Mean 10 cm Soil T (°C)	-	-	-	-	14.4	16.7	-	-	-	15.6
Farm 11 (2021 data)										
Rainfall (mm)	-	-	-	-	74.9	142.1	-	-	-	217.0
LTA (mm)	-	-	-	-	71.1	86.1	-	-	-	157.2
Mean 10 cm Soil T (°C)	-	-	-	-	17.7	15.8	-	-	-	16.8
Pasture Grazing Trials										
Pasture site 1 (2021 data)										
Rainfall (mm)	-	-	-	95.7	29.8	116.3	113.5	-	-	355.3
LTA (mm)	-	-	-	75.3	79.6	86.5	107.8	-	-	349.2
Mean 10 cm Soil T (°C)	-	-	-	10.7	14.9	15.1	16	-	-	14.2
Pasture site 2 (2021 data)										
Rainfall (mm)	-	-	-	135.6	26	97.8	90.6	-	-	350.0
LTA (mm)				64.8	69.8	65.9	82.0			
Mean 10 cm Soil T (°C)	-	-	-	11.5	15.4	17.8	16.4	-	-	15.3
LTA (°C)				12.1	15.1	16.6	16.1			

Figures for LTA (long term averages) are included where available. Figures are only included for the months relevant to the trials

Supplementary Table S5: Total Dry Matter (kg ha⁻¹) of 6 rotations according to year and soil type from the lysimeter trials

	100 % N	75 % N	75 % N + 0.5x PSI-362	75 % N + 1x PSI-362	75 % N + 1.5x PSI-362
2020 Loam	6624 ± 268	5022 ± 394	5889 ± 222	6343 ± 399	6729 ± 426
2020 SL	7011 ± 355	5338 ± 253	6097 ± 432	6750 ± 268	7264 ± 419
2021 Loam	10112 ± 769	8372 ± 480	8181 ± 756	8771 ± 229	9659 ± 638
2021 SL	9606 ± 372	7481 ± 382	8395 ± 747	10070 ± 674	10387 ± 1195

For Loam soils n = 12; for silt-loam soils n = 24. Numbers represent mean ± SE

Supplementary Table S6: Dry matter (kg ha⁻¹) values for the individual farms from the silage trials

Regime	First Cut Silage Trials					
Site	<u>Farm 6</u>	<u>Farm 7</u>				
100 % N	5050	5600				
80 % N + PSI-362	5120	5850				
Regime	Second Cut Silage Trials					
Site	<u>Farm 8</u>	<u>Farm 9</u>	<u>Farm 10</u>			
100 % N	5100	3998	4138			
80 % N + PSI-362	4980	4069	5167			
Regime	Third Cut Silage Trials					
Site	<u>Farm 1</u>	<u>Farm 2</u>	<u>Farm 3</u>	<u>Farm 4</u>	<u>Farm 5</u>	<u>Farm 11</u>
100 % N	5216	4200	3556	3529	4320	4480
80 % N + PSI-362	4992	3984	3388	3600	4248	4530
100 % N + PSI-362	6021	4488	4200	4200	NA	NA

Supplementary Table S7: Daily grass growth (kg DM ha⁻¹ day⁻¹) for the two pasture grazing sites. Each value represents the average of 5 grazings per field.

Pasture Site 1		Pasture Site 2	
100 % N	75 % N + PSI-362	100 % N (PU)	75 % N + PSI-362

Field 1	62.0	Field 2	61.3	Field 1	64.8	Field 2	72.1
Field 3	55.0	Field 4	57.0	Field 3	120.0	Field 4	126.7
Field 5	59.2	Field 6	50.5				
Field 7	54.3	Field 8	71.1				
Field 9	83.4	Field 10	51.5				
Field 11	58.6	Field 12	56.6				
Field 13	59.8	Field 14	82.7				
Field 15	59.8	Field 16	40.6				
Field 17	75.9	Field 18	63.4				
Field 19	58.6	Field 20	57.6				
Field 21	57.5	Field 22	53.2				
Field 23	46.3	Field 24	51.3				
Field 25	46.3	Field 26	62.7				
Field 27	73.1	Field 28	64.9				
Field 29	68.2	Field 30	67.2				
Field 31	64.9	Field 32	65.6				
Field 33	47.1	Field 34	51.0				
Field 35	66.7						

PU = protected urea