Plant production and community structure in a mesic semi-natural grassland: Moderate soil textural variation has a much stronger influence than experimentally increased atmospheric nitrogen deposition

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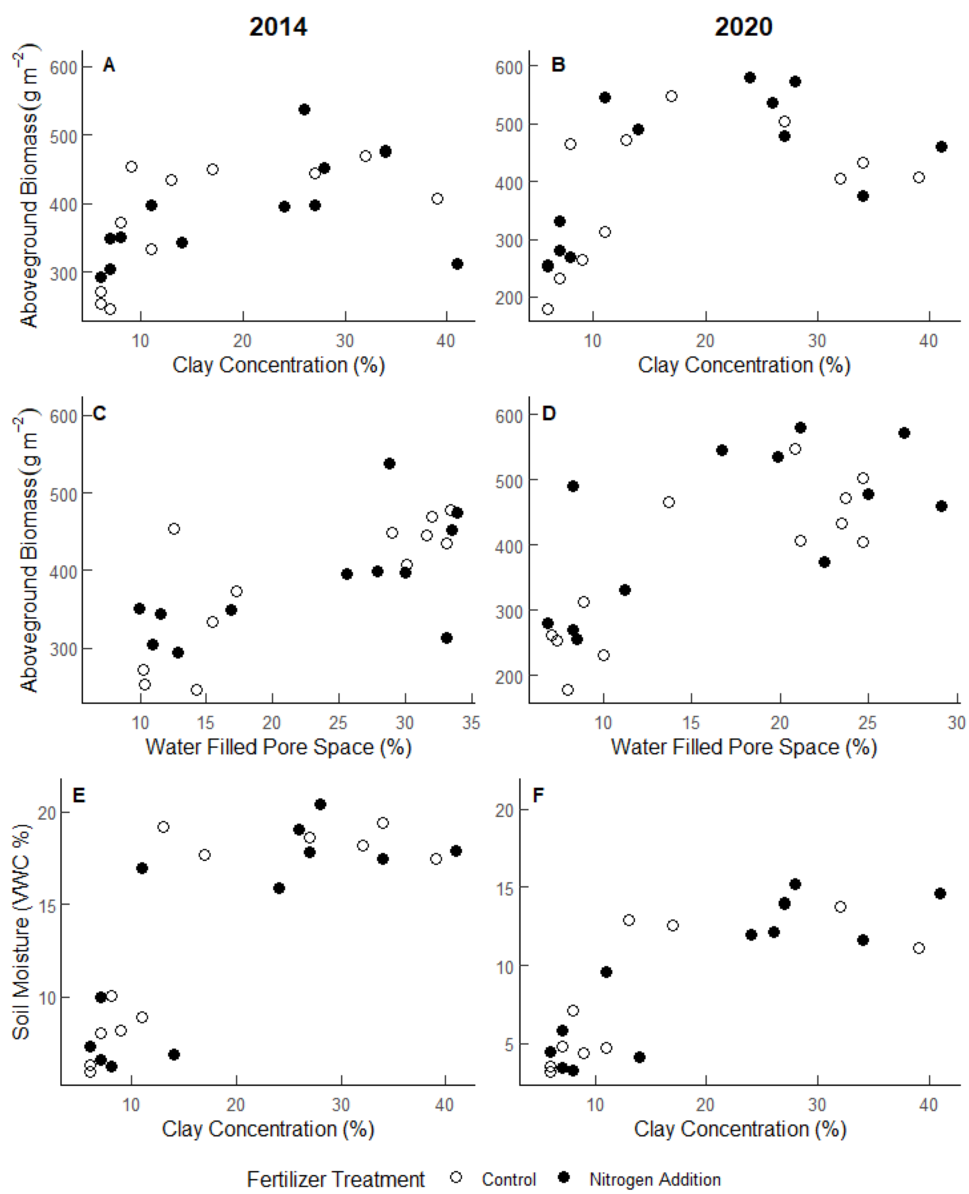


Fig. S1 Total plant community aboveground biomass (g dry mass m^{-2}) in relation to soil clay concentration in 2014 (A) and 2020 (B), mean growing season soil water-filled pore space in 2014 (C) and 2020 (D), and mean growing season soil moisture in relation to mean plot clay concentration in 2014 (E) and 2020 (F) at Stoke's Field hay grassland in each of the twelve chronic low-level N addition (filled circles) and control (open circles) plots. Plot soil moisture (volumetric water content over the interval from 0-5 cm depth) are averages of measurements of five sampling days within each of the 2014 and the 2020 growing seasons.

Fig. S2 Typical soil profiles in the northern sandy-loam (A) and southern predominantly clay-loam (B) sections of Stoke's Field.

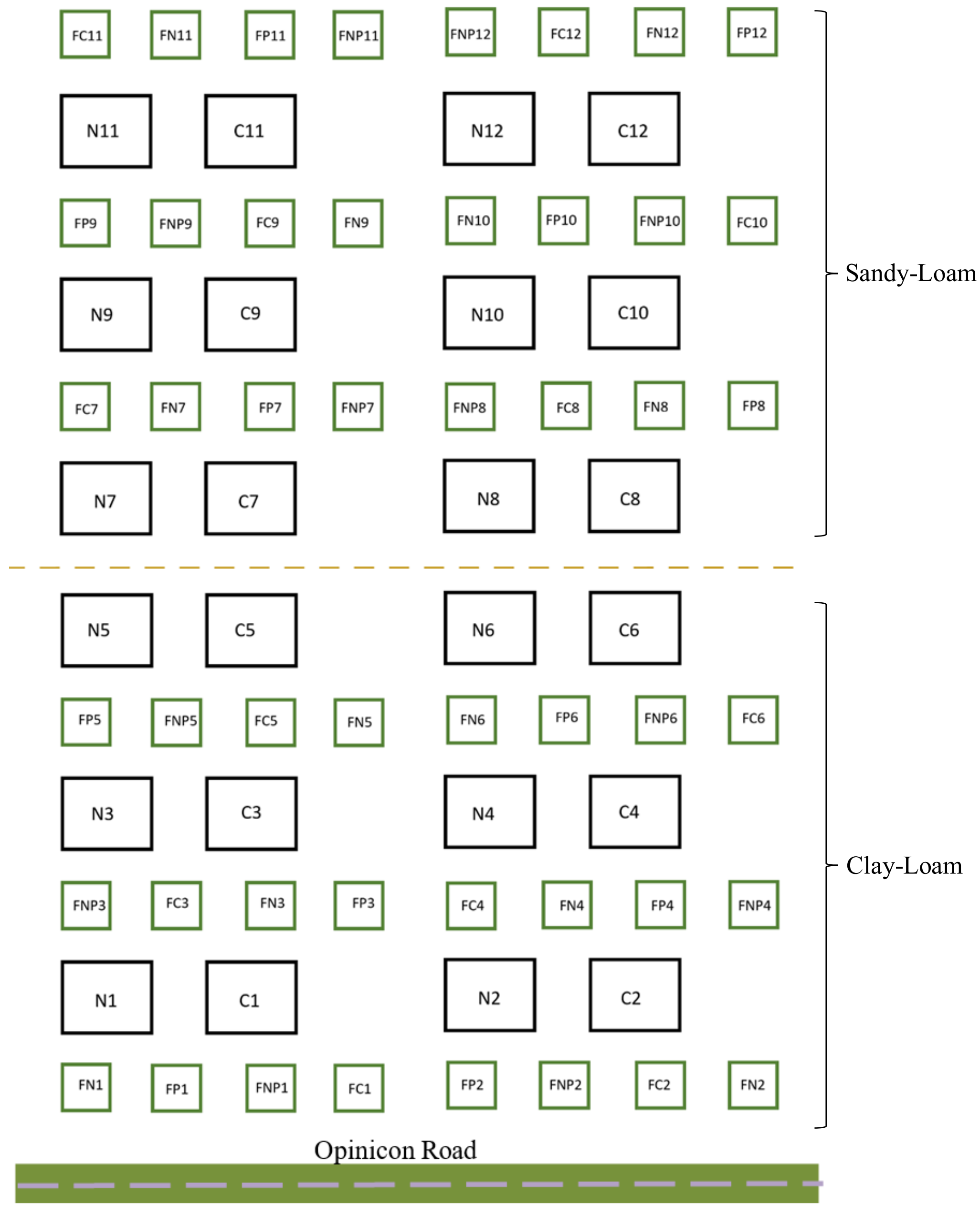


Fig. S3 Plot layout at Stoke's Field for the long-term chronic low-level N addition experiment (black rectangles; 4 x 5 m; 'N' = low-level N addition, 'C' = control), and the separate single-year factorial (F) high-level N + P addition experiment (green squares; 2 x 2 m; 'FN' = high-level N addition, 'FP' = high-level P addition, 'FNP' = high-level N+P addition, 'FC' = control) on both the northern sandy-loam and southern clay-loam sections of the field. Note that the structured random layout of alternate columns of the low-level N addition treatment and the control plots was deliberately designed to account for potential impacts of both east and west-side shading from the tall forest trees bordering each of those sides of the field through the morning and evenings respectively (see Fig 1).

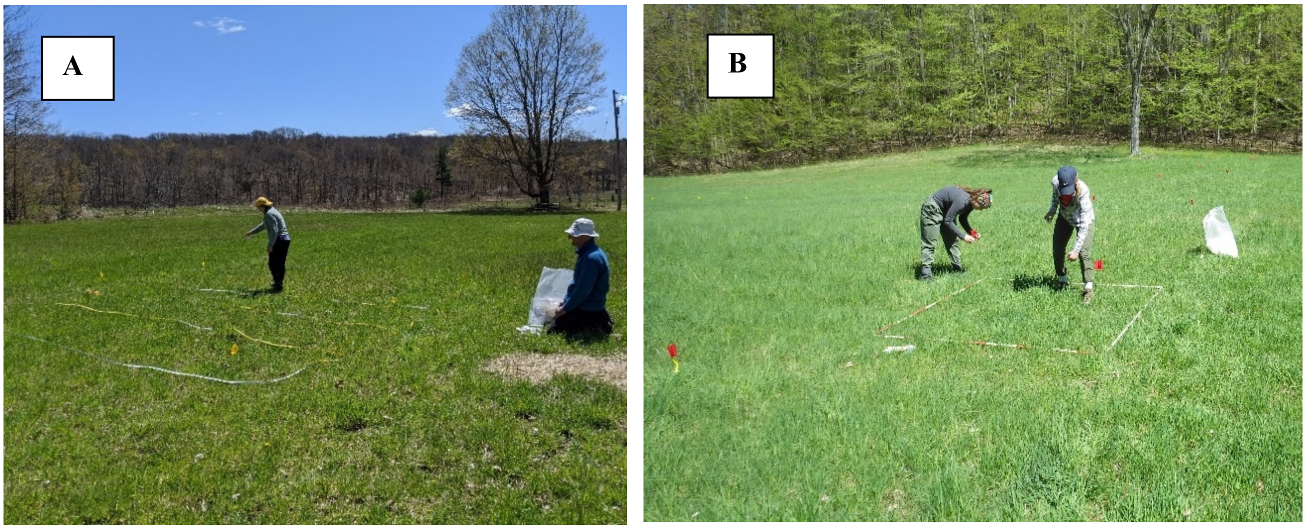


Fig. S4 Fertilizer application procedure for the long term, low-level nitrogen addition experiment (A) and for the separate, single year, factorial high-level nitrogen and phosphorus addition experiment (B).

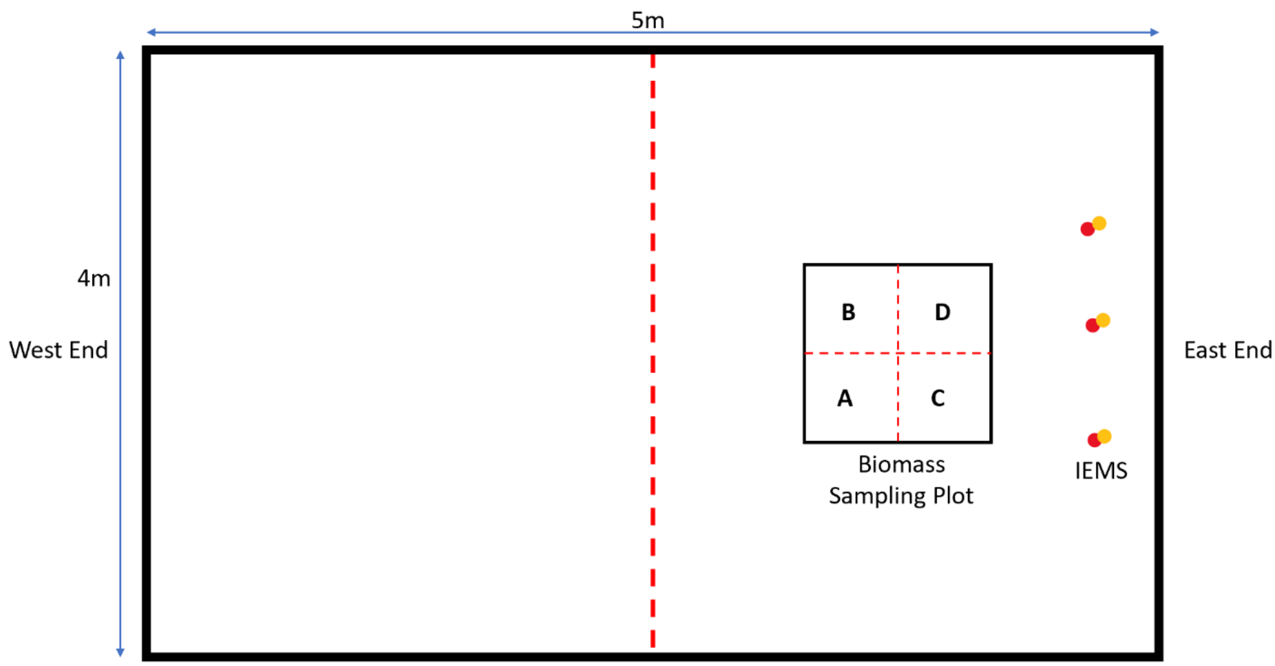


Fig. S5 Plot layout of long-term, low-level nitrogen addition experiment showing the biomass sampling area and the location of the soil cation and anion exchange membrane pairs (IEMs).

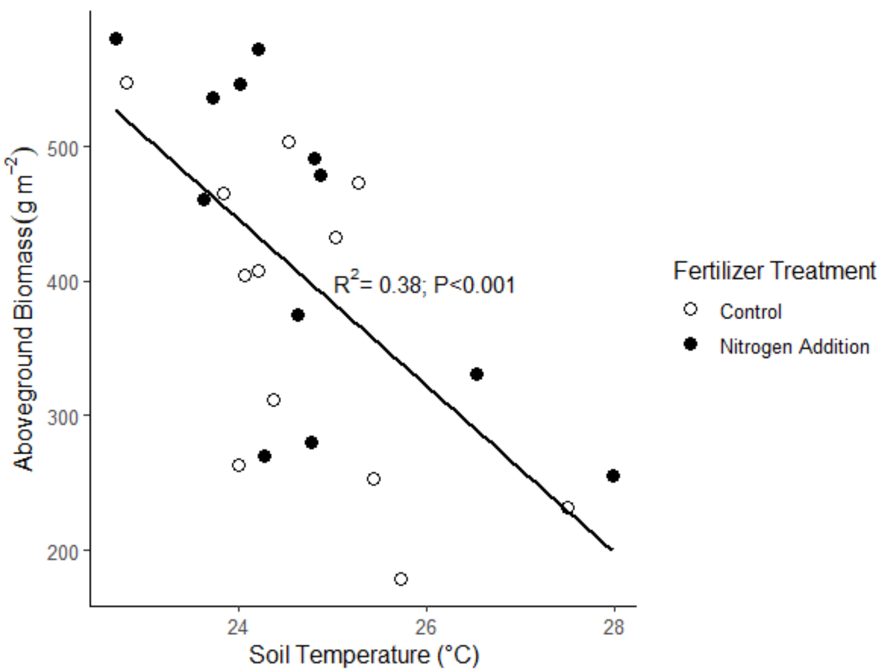


Fig. S6 Total plant community aboveground biomass (g dry mass m^{-2}) in relation to mean growing season daytime soil temperature (measurements at 5 cm depth on five sampling days across the summer of 2020) in the low-level N addition (filled circles) and control (open circles) plots (n = 12) at Stoke's Field hay grassland.

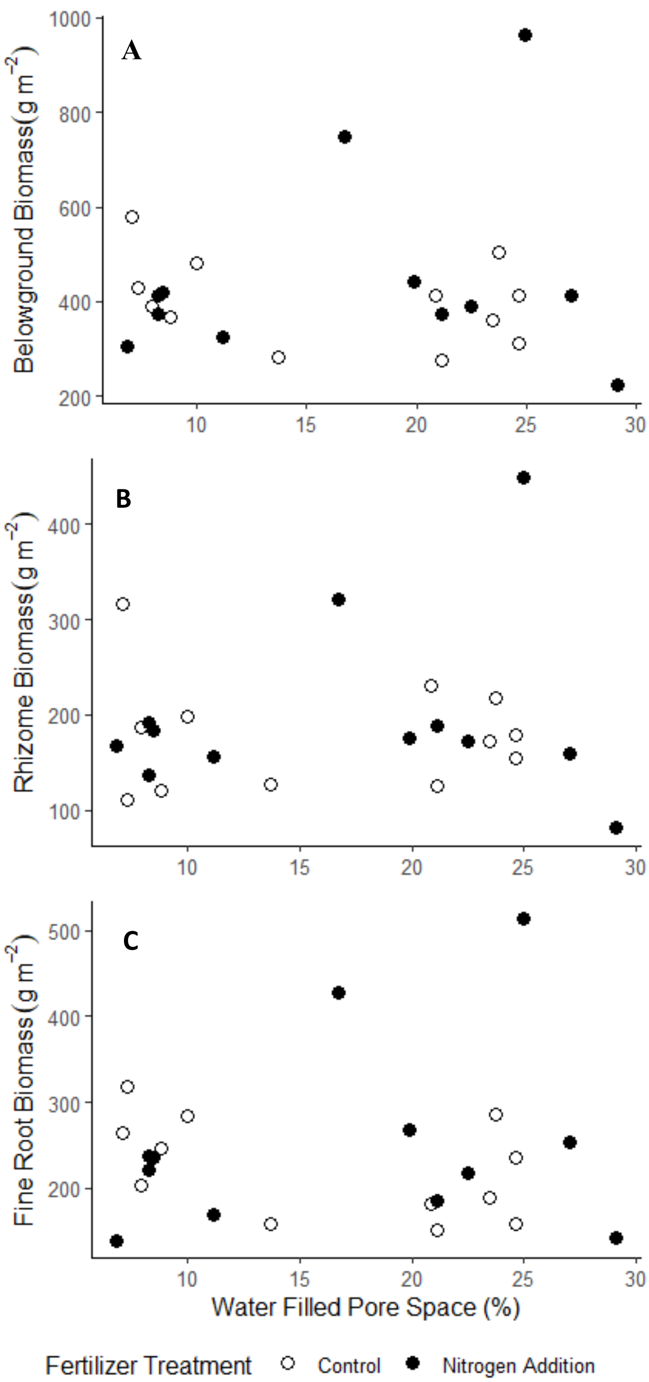


Fig. S7 Total plant community belowground biomass (g dry mass m^{-2}) (A), total plant community rhizome biomass (B), and total plant community fine root biomass (C) in relation to mean growing season soil water-filled pore space at Stoke's Field hay grassland in the summer of 2020 in the N addition (filled circles) and control (open circles) plots (n = 12).

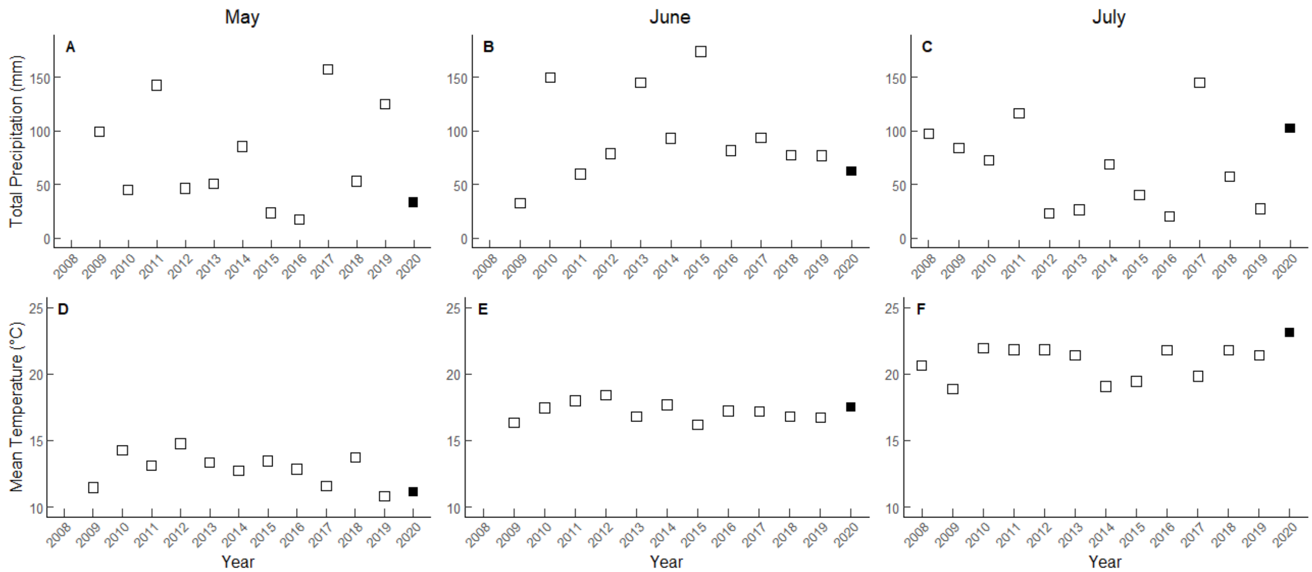
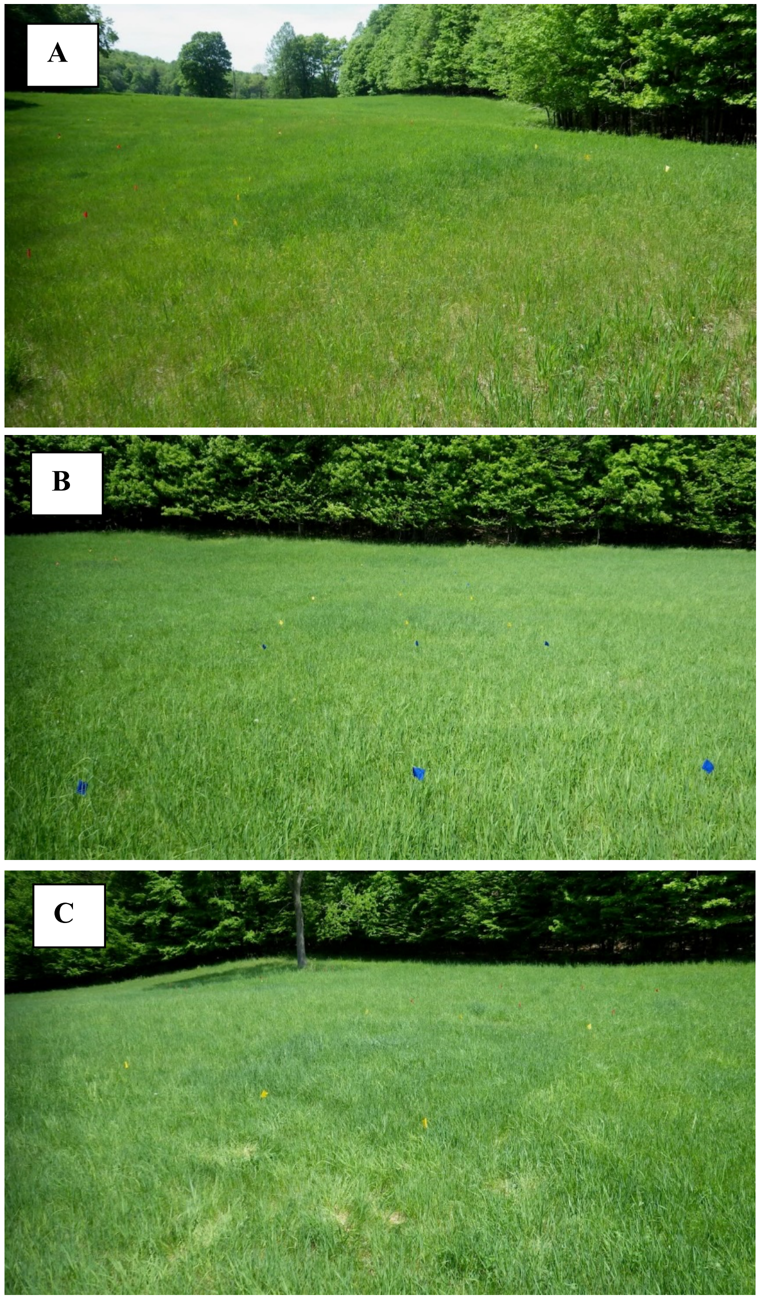


Fig. S8 Total May, June, and July monthly rainfall inputs (mm) (A-C, respectively) and monthly mean air temperatures (°C) (D-F, respectively) from 2008 – 2019 (open squares) and for the 2020 harvest year (filled squares). Data obtained from Environment Canada climate records for the Kingston Climate station (2008-2020) which is ~ 50 km south of Stoke's hayfield.


A
B
C

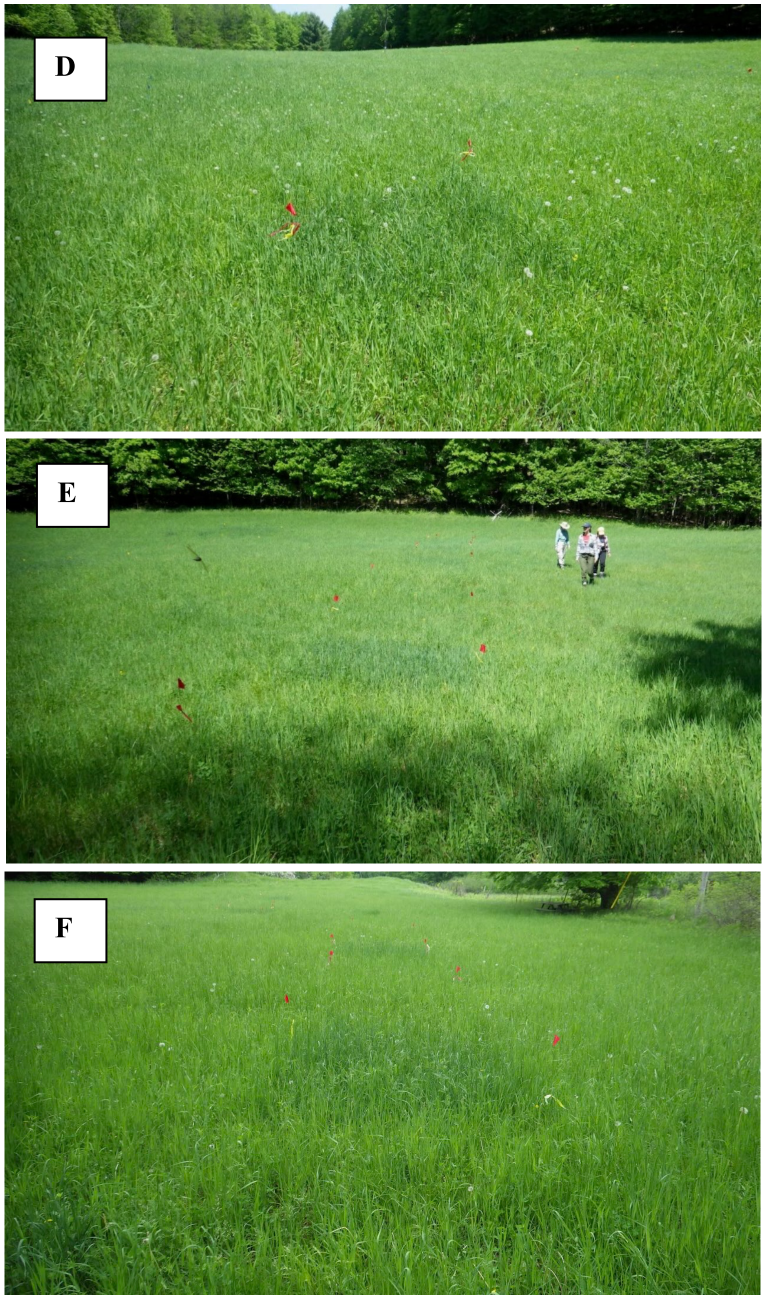


Fig. S9 Photos illustrating the greener shoots of most vegetation in the low-level N addition plots (A, B, C; blue flags- controls, yellow flags- low N addition) and the high-level N+P plots (D, E, F; red+yellow flag – N+P) seven days after the May 20th 2020 fertilizer treatment additions at Stoke's Field.

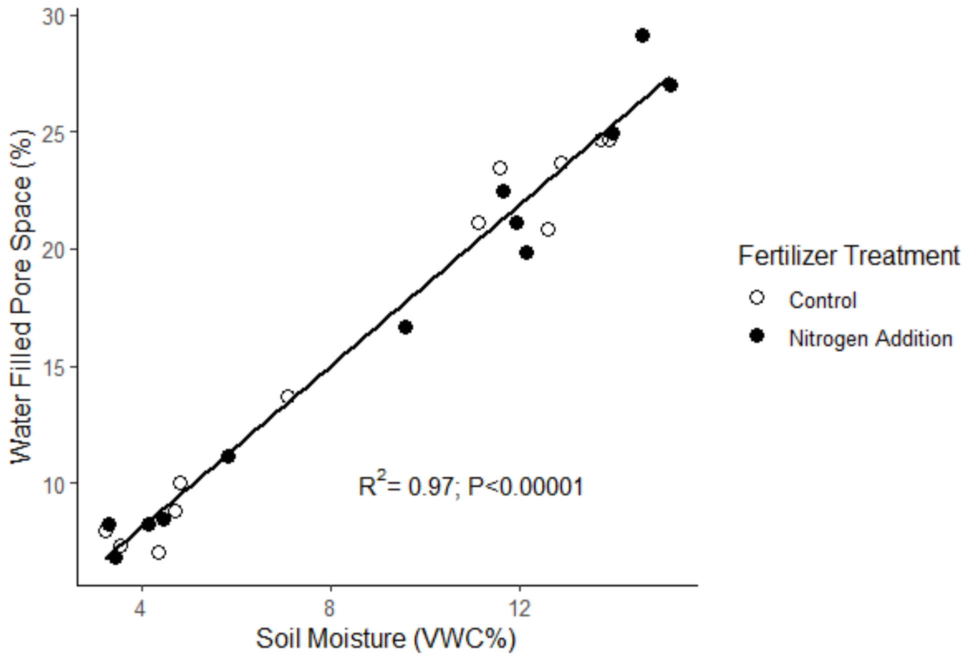


Fig. S10 Mean growing season soil moisture in relation to mean growing season soil water-filled pore space in the N addition (filled circles) and control (open circles) plots (n = 12).

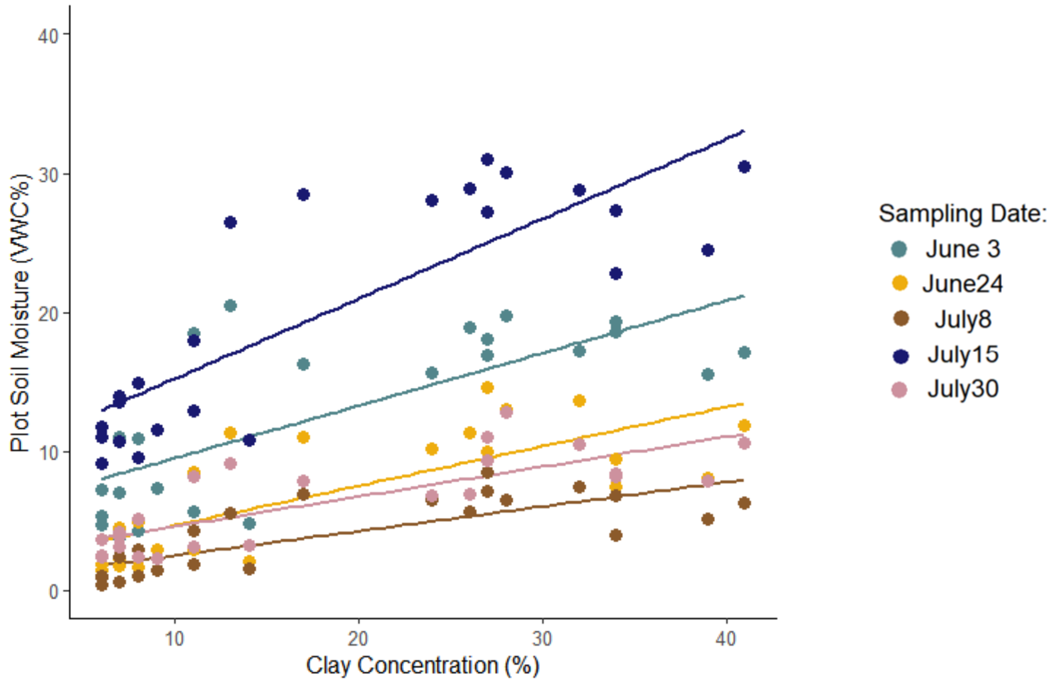


Fig. S11 Mean plot soil moisture (VWC% across 0-5 cm depth) averaged across five sampled dates in the 2020 growing season in relation to soil clay concentration (%) at Stoke's Field. Soil moisture was measured at three random locations within each plot on 5 sampling dates over June and July. Plot-level measurements were averaged for all analyses. The relationship between plot soil moisture and clay concentration on each sampling date is overlain by a regression line that best fits the data (June 3: R^2 = 0.52 , p = < 0.0001 ; June 24: $R^2 = 0.54$, $p < 0.00001$; July 8: $R^2 = 0.59$, $p < 0.00001$; July 15: $R^2 = 0.67$, $p < 0.00001$; July 30: $R^2 = 0.60$, $p < 0.00001$).

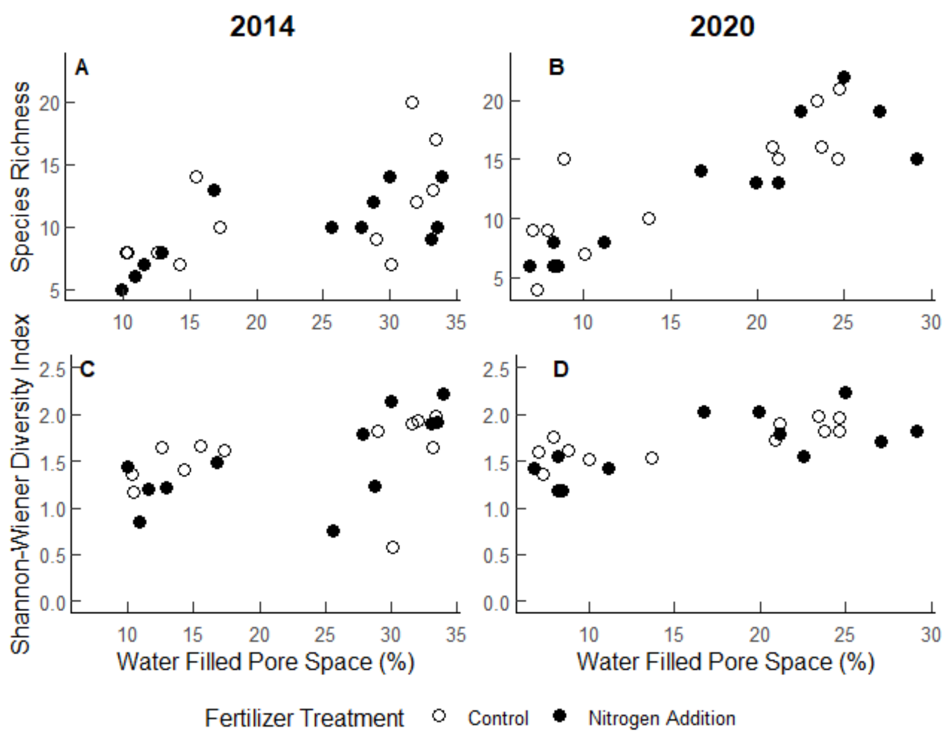


Fig. S12 Plant species richness (A) and species diversity (Shannon-Wiener Diversity Index) (B) in relation to mean growing season soil water-filled pore space in each of the twelve chronic low-level N addition (filled circles) and control (open circles) plots at Stoke's Field hay grassland in the summer of 2014 and 2020.

Table S1. Soil particle size analysis data for each control and chronic low-level N addition plot within the Stoke's Field experiment was collected in the Fall of 2008 except for plot C1 which was collected in 2021 along with five test re-analyses of plots from the 2008 dataset (to ensure precision in methodology across the two datasets). Soil textural categorisation (based on particle size fractions using the U.S.D.A classification system) indicated that the average soil texture composition in the northern half of the field is 'sandy loam' (mean overall soil composition: 8% clay, 22% silt, 70% sand) while the southern half is 'clay loam' (mean soil composition: 29% clay, 43% silt, 28% sand).

Plot	%Clay	%Silt	%Sand
Sandy Loam (Control plots)			
C7	8	22	71
C8	11	22	66
C9	7	23	70
C10	9	21	70
C11	6	19	75
C12	6	20	74
Mean (Standard Error)	**8** (0.8)	**21** (0.6)	**71** (1.3)
Sandy Loam (N addition plots)			
N7	11	13	76
N8	14	28	58
N9	7	22	71
N10	7	24	69
N11	6	21	74
N12	8	27	65
Mean (Standard Error)	**9** (1.2)	**22** (2.2)	**69** (2.7)
Clay Loam (Control plots)			
C1	13	58	28
C2	17	55	28
C3	39	43	18
C4	27	41	32
C5	34	49	18
C6	32	28	39
Mean (Standard Error)	**27** (4.1)	**46** (4.4)	**27** (3.3)
Clay Loam (N addition plots)			
N1	28	41	31
N2	24	48	28
N3	41	44	15
N4	27	39	34
N5	34	41	25
N6	26	31	43
Mean (Standard Error)	**30** (2.6)	**41** (2.3)	**29** (3.8)

Table S2. Model parameters used for full models prior to model selection for each response variable were first measured in relation to the N addition treatment, variation in clay concentration, soil moisture, soil temperature, and the interaction between N addition treatment and clay concentration (denoted as *). In terms of potential correlation among explanatory variables, we compared the fit of soil moisture, soil clay concentration, and WFPS in all models since these three properties are inter-related and found the latter was the best predictor. We therefore, reanalyzed the data replacing soil moisture and clay concentration variables with water filled pore space (WFPS) and reported the WFPS-only analyses in the manuscript Results. The models for aboveground biomass and species diversity were evaluated using linear models. The model for species count was evaluated using a generalized linear model fitted with a Poisson distribution. The models for belowground biomass and its component measurements, as well as ammonium were evaluated using a generalized linear model fitted with an "inverse gaussian" transformation. The models for nitrate and phosphate were evaluated using a generalized linear models fitted with a "gamma" transformation and an inverse link ($g(\mu) = 1/\mu$). Full model prior to model selection for both iterations of the analysis are presented here. Results of statistical tests for each full model were obtained using 'summary' and 'Anova' functions in R on each model.

Dependent Variable	Full Model Predictor Variables	Df	F-Value	P-Value
Total Shoot Biomass (g/m^2)	Low-level N Addition	1,19	2.76	0.11
	WFPS	**1,19**	**16.26**	**0.00071**
	Low-level N Addition*WFPS	1,19	1.48	0.24
	Soil Temperature	**1,19**	**9.15**	**0.007**
Total Shoot Biomass (g/m^2)	Low-level N Addition	1,18	4.15	0.057
	Clay Content	**1,18**	**5.00**	**0.038**
	Soil Moisture	**1,18**	**26.18**	**>0.00001**
	Low-level N Addition*Clay Content	1,18	1.49	0.24
	Soil Temperature	**1,18**	**10.67**	**0.0043**
Total Belowground Biomass (g/m^2)	Low-level N Addition	1,19	0.64	0.43
	WFPS	1,19	0.17	0.69
	Low-level N Addition*WFPS	1,19	1.38	0.26
	Soil Temperature	1,19	0.37	0.55
Total Belowground Biomass (g/m^2)	Low-level N Addition	1,18	1.27	0.27
	Clay Content	**1,18**	**4.65**	**0.045**
	Soil Moisture	**1,18**	**4.51**	**0.048**
	Low-level N Addition*Clay Content	1,18	0.49	0.50
	Soil Temperature	1,18	0.21	0.65
Fine Root Biomass (g/m^2)	Low-level N Addition	1,19	0.68	0.42
	WFPS	1,19	0.2	0.66
	Low-level N Addition*WFPS	1,19	2.78	0.11
	Soil Temperature	1,19	0.95	0.34
Fine Root Biomass (g/m^2)	Low-level N Addition	1,18	1.87	0.29
	Clay Content	1,18	3.52	0.077
	Soil Moisture	1,18	3.52	0.077
	Low-level N Addition*Clay Content	1,18	1.58	0.22
	Soil Temperature	1,18	0.69	0.42
Rhizome Biomass (g/m^2)	Low-level N Addition	1,19	0.38	0.54
	WFPS	1,19	0.088	0.77
	Low-level N Addition*WFPS	1,19	0.19	0.67
	Soil Temperature	1,19	0.0083	0.93
Rhizome Biomass (g/m^2)	Low-level N Addition	1,18	0.78	0.39
	Clay Content	1,18	3.59	0.074
	Soil Moisture	1,18	3.46	0.079
	Low-level N Addition*Clay Content	1,18	0.0009	0.98
	Soil Temperature	1,18	0.0009	0.98
Species Richness	Low-level N Addition	1,19	1.22	0.28
	WFPS	**1,19**	**40.13**	**>0.00001**
	Low-level N Addition*WFPS	1,19	0.12	0.73
	Soil Temperature	1,19	0.19	0.67

Table S2 Continued.

Species Richness	Low-level N Addition	1,18	1.27	0.27
	Clay Content	1,18	0.41	0.53
	Soil Moisture	**1,18**	**13.69**	**0.0016**
	Low-level N Addition*Clay Content	1,18	0.011	0.92
	Soil Temperature	1,18	0.0001	0.99
Species Diversity (Shannon-Wiener Diversity Index)	Low-level N Addition	1,19	0.92	0.35
	WFPS	**1,19**	**13.88**	**0.0014**
	Low-level N Addition*WFPS	1,19	0.38	0.54
	Soil Temperature	1,19	0.86	0.37
Species Diversity (Shannon-Wiener Diversity Index)	Low-level N Addition	1,18	0.91	0.35
	Clay Content	1,18	0.0072	0.93
	Soil Moisture	**1,18**	**5.93**	**0.026**
	Low-level N Addition*Clay Content	1,18	0.11	0.75
	Soil Temperature	1,18	0.65	0.43
Ammonium Flux (μg/cm^2/day)	Low-level N Addition	1,19	0.76	0.39
	WFPS	**1,19**	**38.23**	**>0.00001**
	Low-level N Addition*WFPS	1,19	0.0061	0.94
	Soil Temperature	1,19	0.11	0.74
Ammonium Flux (μg/cm^2/day)	Low-level N Addition	1,18	2.56	0.13
	Clay Content	**1,18**	**16.64**	**0.0007**
	Soil Moisture	1,18	1.80	0.20
	Low-level N Addition*Clay Content	1,18	0.13	0.72
	Soil Temperature	**1,18**	**4.36**	**0.051**
Nitrate Flux (μg/cm^2/day)	Low-level N Addition	1,19	1.89	0.18
	WFPS	**1,19**	**24.62**	**>0.00001**
	Low-level N Addition*WFPS	1,19	0.6	0.45
	Soil Temperature	1,19	0.29	0.6
Nitrate Flux (μg/cm^2/day)	Low-level N Addition	1,18	1.39	0.25
	Clay Content	1,18	1.72	0.21
	Soil Moisture	1,18	3.18	0.092
	Low-level N Addition*Clay Content	1,18	0.50	0.49
	Soil Temperature	1,18	0.053	0.82
Phosphate Flux (μg/cm^2/day)	Low-level N Addition	1,19	0.93	0.35
	WFPS	1,19	1.68	0.21
	Low-level N Addition*WFPS	1,19	4.14	0.056
	Soil Temperature	1,19	0.42	0.53
Phosphate Flux (μg/cm^2/day)	Low-level N Addition	1,18	1.20	0.29
	Clay Content	1,18	2.75	0.11
	Soil Moisture	1,18	0.38	0.54
	Low-level N Addition*Clay Content	1,18	2.81	0.11
	Soil Temperature	1,18	0.019	0.89

Table S3. Results of the non-parametric Kruskal-Wallis test of several combinations of above to belowground biomass ratios between soil texture and N addition treatment. Plot textures were grouped into two separate categories based on clay content and labelled as clay-loam or sandy-loam.

Biomass Ratios	Predictor Variable	Chi-Squared	df	P-Value
Above: Belowground Ratio	**Soil Texture**	**6.8**	**1**	**0.009**
Above: Belowground Ratio	Low-level N Addition	0.2	1	0.6
Above: Fine Root Ratio	**Soil Texture**	**6.8**	**1**	**0.009**
Above: Fine Root Ratio	Low-level N Addition	0.3	1	0.6
Above: Rhizome Ratio	**Soil Texture**	**4.08**	**1**	**0.04**
Above: Rhizome Ratio	Low-level N Addition	0.003	1	0.7
Rhizome: Fine Root Ratio	Soil Texture	0.2	1	0.7
Rhizome: Fine Root Ratio	Low-level N Addition	0.2	1	0.6

Table S4. Mean values of total plant community aboveground biomass, total belowground biomass and its individual components, species richness, species diversity (Shannon-Wiener Diversity Index), soil clay and sand concentrations, soil bulk density, mean growing season soil water-filled pore space (WFPS), soil moisture (volumetric water content), soil temperature, and mid-July soil nutrient fluxes for the control and chronic low-level N addition treatment plots (n = 6, standard errors in parentheses) in the clay-loam and sandy-loam sections of Stoke's hayfield experimental site in 2014 and 2020.

Soil Property	Clay-Loam				Sandy-Loam			
	Control		N Addition		Control		N Addition	
	2014	2020	2014	2020	2014	2020	2014	2020
Total Shoot Biomass (g m^{-2})	447.2 (25)	461.4 (23)	428.4 (77)	500.5 (32)	322.2 (81)	284.2 (40)	340.1 (38)	362.3 (51)
Total Belowground Biomass (g m^{-2})		379.8 (33)		467.7 (104)		421.8 (41)		431.1 (66)
Fine Root Biomass (g m^{-2})		199.8 (21)		263.2 (54)		245.2 (24)		238.1 (41)
Rhizome Biomass (g m^{-2})		180.1 (16)		204.5 (52)		176.6 (32)		193.0 (27)
Species Richness	13 (5)	17.2 (1)	12 (2)	16.8 (2)	9 (3)	9.0 (1)	8 (3)	8.0 (1)
Species Diversity	1.6 (0.5)	1.9 (0.04)	1.7 (0.6)	1.9 (0.1)	1.5 (0.2)	1.6 (0.05)	1.3 (0.3)	1.5 (0.1)
Soil Clay Content (%)	27.0 (4)	27.0 (4)	30.0 (3)	30.0 (3)	7.8 (0.8)	7.8 (0.8)	8.8 (1)	8.8 (1)
Soil Sand Content (%)	27.2 (3)	27.2 (3)	29.3 (4)	29.3 (4)	71.0 (1)	71.0 (1)	68.8 (3)	68.8 (3)
Soil Bulk Density (g cm^{-3})	1.10 (0.02)	1.10 (0.02)	1.09 (0.06)	1.09 (0.06)	1.08 (0.04)	1.08 (0.04)	1.06 (0.02)	1.06 (0.02)
Soil Moisture (VWC%)	18.4 (0.8)	12.6 (0.5)	18.1 (2)	13.3 (0.6)	7.9 (2)	4.6 (0.6)	9.0 (4)	5.1 (1)
Soil Water Filled Pore Space (%)		23.1 (2)		24.1 (4)		9.2 (2)		10.0 (4)
Soil Temperature (°C)		24.3 (0.4)		24.0 (0.3)		25.2 (0.6)		25.4 (0.6)
Soil Ammonium (μg cm^{-2} day^{-1})		93.6 (11)		89.5 (8)		211.9 (37)		257.1 (42)
Soil Nitrate (μg cm^{-2} day^{-1})		24.0 (4)		19.3 (4)		81.9 (18)		51.4 (18)
Soil Phosphate (μg cm^{-2} day^{-1})		9.4 (2)		16.9 (6)		22.7 (6)		23.3 (4)

Table S5. Total plant community shoot nitrogen concentrations (%) and pools (g N m^{-2}) in the low-level N addition treatment and control plots on the predominantly clay-loam and sandy-loam sections of Stoke's field in July 2014. Data are means and standard errors (n=6), and statistical analysis was by two-way ANOVA. Total N concentrations (% of dry mass) of plant community shoot tissue were analysed by combustion and gaseous N detection (Elementar, Hanau, Germany), and shoot nitrogen pools for each plot were calculated by multiplying the N concentration by the total above-ground biomass.

Sandy Loam (Control plots)	**Shoot Nitrogen Concentration (% dw)**	**Total Shoot Nitrogen Pool (g m^{-2})**
c7	1.09	4.05
c8	1.56	5.19
c9	1.49	3.66
c10	1.72	7.83
c11	1.52	4.14
c12	1.11	2.83
Mean (Standard Error)	**1.17** (0.10)	**4.62** (0.71)
Sandy Loam (N addition plots)		
n7	1.3	5.2
n8	1.44	4.95
n9	1.14	3.98
n10	1.65	5.03
n11	1.51	4.42
n12	1.81	6.35
Mean (Standard Error)	**1.48** (0.10)	**4.99** (0.33)
Clay Loam (Control plots)		
c1	1.11	4.83
c2	1.07	4.82
c3	1.68	6.84
c4	0.96	4.29
c5	1.12	5.34
c6	1.09	5.1
Mean (Standard Error)	**1.17** (0.10)	**5.2** (0.36)
Clay Loam (N addition plots)		
n1	1.01	4.55
n2	0.95	3.77
n3	1.2	3.75
n4	0.92	3.7

n5	1.09	5.16
n6	1.06	5.69
Mean (Standard Error)	**1.04** (0.06)	**4.44** (0.26)

Two-Way ANOVA Results

Variable	Shoot Nitrogen Concentration (% dw)			Total Shoot Nitrogen Pool (g m^{-2})		
	df	**F-Value**	**P-Value**	**df**	**F-Value**	**P-Value**
Soil Texture	1,20	13.84	**0.0013**	1,20	0.0014	0.97
Treatment	1,20	0.161	0.69	1,20	0.18	0.68
Soil Texture x Treatment	1,20	1.13	0.30	1,20	1.50	0.24

Table S6. Soil microbial biomass carbon and nitrogen pools (0-10 cm depth) in the low-level N addition treatment and control plots on the predominantly clay-loam and sandy-loam sections of Stoke's Field in October 2008. Data are means and standard errors (n=6), and statistical analysis was by two-way ANOVA. Soil microbial C and N were measured using the chloroform fumigation direct-extraction method (Brookes et al. 1985).

Sandy Loam (Control plots)	**Microbial Biomass Carbon (g m^{-2})**	**Microbial Biomass Nitrogen (g m^{-2})**
c7	30.11	6.82
c8	42.58	9.10
c9	28.88	6.28
c10	41.05	8.79
c11	30.49	6.39
c12	26.69	5.82
Mean (Standard Error)	**33.30** (2.8)	**7.20** (0.6)
Sandy Loam (N addition plots)		
n7	26.28	6.41
n8	36.17	7.70
n9	35.17	7.10
n10	28.50	5.82
n11	24.11	6.02
n12	52.09	11.28
Mean (Standard Error)	**33.72** (4.2)	**7.39** (0.8)
Clay Loam (Control plots)		
c1	62.71	14.07
c2	57.89	13.41
c3	91.90	20.89
c4	73.54	15.95
c5	87.79	17.73
c6	77.45	16.30
Mean (Standard Error)	**75.21** (5.5)	**16.39** (1.1)
Clay Loam (N addition plots)		
n1	70.63	16.03
n2	66.94	14.77
n3	119.12	25.85
n4	69.45	14.10

n5	93.97	18.60
n6	90.86	17.40
Mean (Standard Error)	**85.16** (8.3)	**17.79** (1.7)

Two-Way ANOVA Results

Variable	Microbial Biomass Carbon (g m^{-2})			Microbial Biomass Nitrogen (g m^{-2})		
	Chi-squared	**df**	**P-value**	**Chi-squared**	**df**	**P-value**
Soil Texture	70.64	1,20	**< 0.0001**	93.16	1,20	**< 0.0001**
Treatment	0.48	1,20	0.5	0.38	1,20	0.54
Soil Texture x Treatment	0.13	1,20	0.72	0.2	1,20	0.66

Table S7. Mean aboveground biomass (g dry mass m^{-2}) of key N-fixing species and all N-fixing species combined in response to the long-term, low-level N addition experiment on the predominantly clay-loam and sandy-loam sections of Stoke's Field (n = 6, standard errors in parentheses) in 2014 and 2020.

		2014				2020			
Soil Texture	**Treatment**	**Cow's Vetch**	**Hop Clover**	**Red Clover**	**Total Legume**	**Cow's Vetch**	**Hop Clover**	**Red Clover**	**Total Legume**
Sandy Loam	Control	3.8 (1.9)	0.0 (0)	0.01 (0.01)	3.8 (1.9)	1.3 (0.5)	0.1 (0.1)	2.2 (0.9)	2.5 (0.9)
	N Addition	3.3 (1.8)	0.0 (0)	0.07 (0.07)	3.4 (1.7)	1.2 (0.6)	0.1 (0.1)	1.5 (0.9)	2.9 (1.2)
Clay Loam	Control	4.9 (1.3)	0.0 (0)	1.1 (0.7)	6.0 (1.5)	2.2 (0.6)	0.2 (0.1)	7.6 (1.4)	10.1 (1.2)
	N Addition	1.8 (0.9)	0.5 (0.5)	0.8 (0.6)	3.1 (1.3)	1.2 (0.4)	1.1 (0.5)	9.2 (2.3)	11.5 (2.4)

Table S8. Specific measures taken by the authors to reduce the environmental impacts of the data collection reported in this study.

Category of Scientific Activity	Details of Activity	Ecological Impacts of Activity	Measures Taken to Reduce those Ecological Impacts
Travel	Fieldwork travel by vehicle to and from the study site.	Greenhouse gasses associated with driving to and from field site.	Field crew car-pooled to the study site whenever possible, and avoided unnecessary travel by extending our data collection days to reduce the number of trips required over the summer.
Project Materials	Flagging for site set-up and materials for nutrient analysis and sample collection were required to successfully conduct data collection.	Increased inputs of non-biodegradable waste to the landfill.	Materials used to mark the plots and study site were re-used from previous experiments. Ion exchange membranes were recycled from a previous experiment, having first checked that ion concentrations in these re-charged membranes were below detectable limits and therefore, appropriate to use for this study. Paper bags were used instead of plastic in all possible circumstances.

REFERENCES:

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837e842.

Protocol S1. Belowground Biomass Sample Processing Protocol

A. Sample Collection

Materials

- Shovel
- Garden clippers
- Garbage bag
- Sharpie and label
- Ruler

Trim all aboveground biomass as close to soil level as possible and remove any leaf litter (Figure 1a). Use a shovel to cut and remove a square section of soil approximately (30 cm width x 30 cm length x 10 cm depth; Figure 1b,c), and place in a plastic bin or on a garbage bag to measure the length, width, as well as the depth at three separate locations. These measurements will be used later to compute the volume and standardize biomass data. Place soil sample in a garbage bag and label accordingly. Freeze until ready to wash and sort the root biomass.

Figure 1. Images depicting the cutting (A), and removal (B-C) of soil sections.

B. Soil Core Washing

Materials

- 3 Plastic Basins
- Serrated Knife
- Scissors
- 2 mm sieve
- Soil-adapted (i.e. non-clogging) sink

Trimming and Prepping

Remove the soil sample from freezer and let thaw for 24 hrs before cutting it in half and soaking each piece in a separate basin for 12 hrs (acceptable soaking time ranges from 8-48 hrs). Drain water from one basin and cut away any remaining aboveground biomass (Figure 2a). Clip away as much of the aboveground biomass and dead material as possible as this will make sorting through the sieve much easier later. The clipped soil section should look completely bare once you have finished removing the plant shoots (Figure 2b). Cut this section in half and remove one of the quarters from the bin to clean later (Figure 2c). Add water as needed to the basin to ensure that the remaining quarter is submerged.

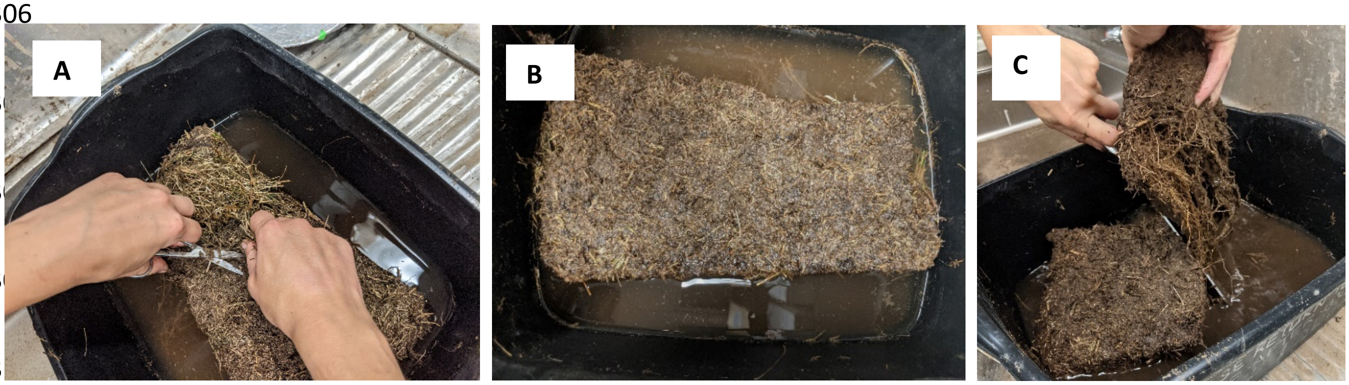


Figure 2. Images depicting trimming aboveground biomass (A), soaking the soil section (B), and cutting the section in half (C).

Washing the Core

Massage the quarter with your fingers to remove as much soil as possible (Figure 3a). If the soil section is very densely packed, you will need to turn the quarter over and massage it from the bottom to top. The roots in the upper 2-3 cm of the core can be packed very tightly and soil can get trapped within them (Figure 3b). To address this issue, slowly work your fingers through, creating little spaces in the soil core to spread the roots out as much as possible.

Once both quarters have been massaged thoroughly and the bulk of the soil is removed from the section, rinse one of the quarters in a new bucket (here on referred to as 'rinse bucket'). Once the quarter is rinsed briefly under the tap (Figure 3c), pour the water trapped in the bucket into the sieve, apart from the bottom residue that contains most of the sediment (this portion can go into the initial 'wash bucket'). Contents of the rinse bucket should always be poured through the sieve to catch any roots that were separated from the quarter that is being sorted (Figure 3d). Spread the quarter out in the rinse bucket and massage/rub the core against the bottom of the bucket as water is pouring into the bucket (Figure 3e). This will allow you to loosen up the core even more. Rinse the core again under the tap and pour all of the water from the rinse bucket into the sieve (except the bottom residue that contains most of the sediment again). Wash out the rinse bucket and repeat the washing again. A third cycle of washing may be required if the roots are woven tightly together.

The quarter is considered clean when the water in the bucket contains little sediment and is quite clear. At this point, the quarter can be rinsed once more and set aside for sorting. Repeat for remaining quarters until you have 4 clear quarters ready to be sorted. To preserve over multiple days, submerge the quarters in water and place in fridge. Whole soil samples should be completely sorted within a week of washing.

Figure 3. Images depicting massaging the root mass to remove soil (A), tightly packed root mass (B), rinsing loose soil from root mass (C), pouring rinse bucket through sieve (D), and massaging root mass against rinse bucket to loosen soil (E).

Sorting the Initial Wash Buckets

Using your hands, skim across the surface of the wash bucket water to remove the dead plant material floating at the top (Figure 4a). This material can be identified as dead by the absence of fine roots and the presence of deteriorated rhizomes (that break apart easily) and dead aboveground biomass (e.g., shoot fragments). Put this material in the garbage.

Work through the mud in the basin by scooping up handfuls and sorting through it with your fingers. Put all contents you find in the sieve for washing later (Figure 4b). The wash bucket has been sufficiently sorted when you are not pulling up any roots and/or are only pulling up very small amounts of fine roots. Keep in mind 'diminishing returns'. At this point the wash bucket has been completely sorted.

Leave the wash bucket for at least 4 hours to let the sediment settle before draining the water into the sink, and disposing of the sediment as solid waste.

Figure 4. Images depicting removal of dead material floating on water surface (A), and sorting through bucket using fingers (B).

Rinsing the Sieve

Turn the tap water on and let it run through the sieve, moving the sieve around and massaging the roots so that most of the mud drains out (Figure 5a). Once the sieve has been rinsed, form the roots into several clumps/balls and put into the rinse bucket. With the water running, rinse the roots using the same motion as used for the soil quarters. Once the rinse bucket is partially full of water, grab a clump of loose roots and form them into a ball (Figure 5b). Rinse this ball under the water flow to remove any soil or debris and place in the sieve. Do this as needed to get a large portion out of the rinse bucket. Then, drain the rinse bucket through the sieve until almost all of the water has been drained (Figure 5c). Check for any remaining rhizomes in the rinse bucket and then pour the rest into the original wash bucket as it is mostly just soil that has been washed from the sieved roots. Repeat this step two more times or until the water is clear when it is being poured into the sieve. Keep sieve contents submerged in water and refrigerated until you are ready to sort.

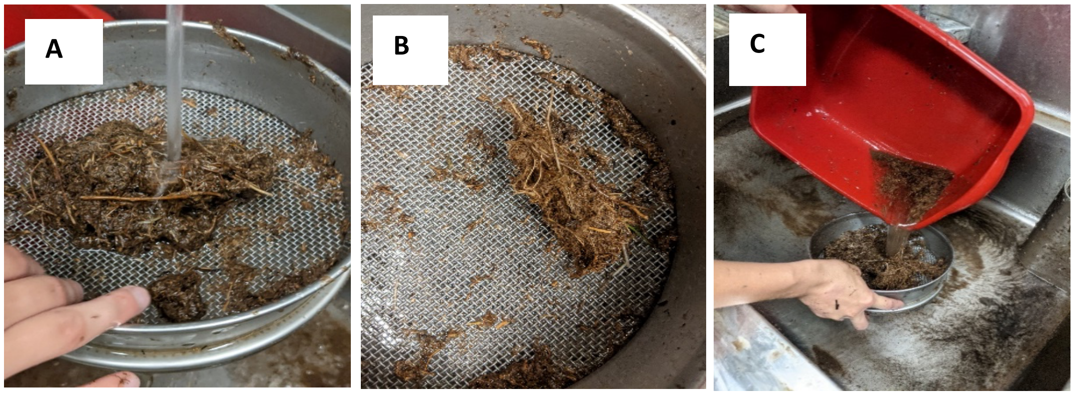


Figure 5. Images depicting rinsing roots in the sieve (A), clumping roots together for further washing (B), and draining rinse bucket through sieve (C).

C. Sorting the Core

Materials

- Pie plates
- Tweezers
- Scissors
- Water

Sorting the Sieve

Remove small chunks of roots from the sieve using tweezers (Figure 6a) and place them on a pie plate that contains water. This will allow the roots to separate and will make it easier to pick out live roots from dead material and debris. Carefully remove the fine roots and live rhizomes and place them in their designated trays. A second pie plate with water may be needed to help rinse and pick out the small sections of roots.

Dead material and debris can either be removed from the pie plate and placed in a third pie plate to be composted later, or it can remain in the pie plate and the water can be replaced periodically (Figure 6b). Water in the sorting pie plate can be replaced by draining the water through the sieve so as to catch the debris that needs to be discarded, then filling the pie plate up with fresh water again. Work through the sieve material until all biomass has been appropriately sorted into either rhizome, fine root, or dead material categories (Figure 6c).

Figure 6. Image depicting clean sieved plant material ready to be sorted (A), pie plates containing dead biomass and debris (B), and sorted fine roots and rhizomes (C).

Sorting the quarters

Work through each quarter, slowly pulling and teasing apart the fine roots from each other (Figure 7a). When sorting loosely packed quarters, roots should be easy to pull apart without ripping. For tightly packed quarters (typically found on sandy soils), it might not be possible to easily pull them apart and some tearing will occur. Separate the rhizomes from the fine roots and place them in their separate pie plates (Figure 7b). Snip any aboveground biomass from the rhizomes and place in the discarded pile. Also snip or tear away any fine roots attached to the rhizomes and place those in the fine root pile. Note: some material clustered right around certain rhizomes will be dead and should be sorted accordingly. It can be helpful to have a rinsing pie plate during the quarter sorting as well, to help separate the roots from each other and from the remaining debris.

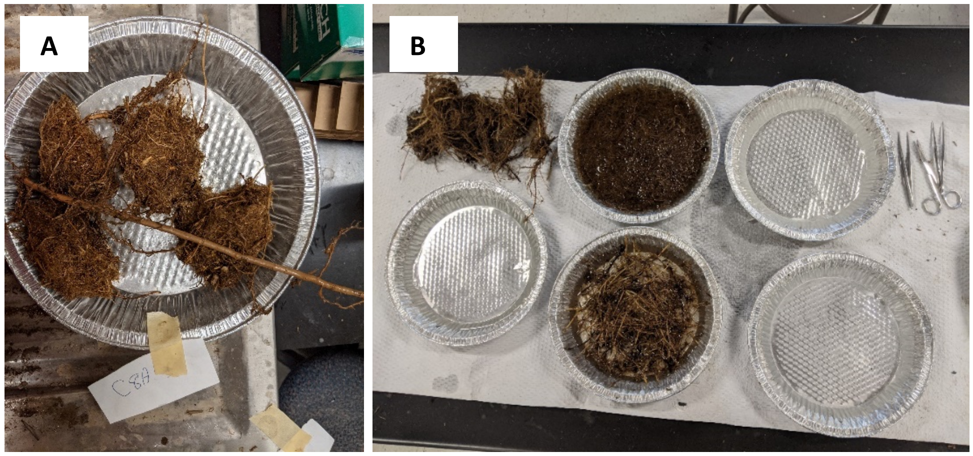


Figure 7. Images depicting washed root quarters ready to be sorted (A), and assortment of pie plates for washing and sorting the root quarters (B).