Supplementary Materials

Biodiversity-Based Empirical Critical Loads of Nitrogen Deposition in the Athabasca Oil Sands Region

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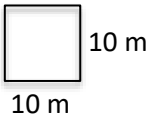
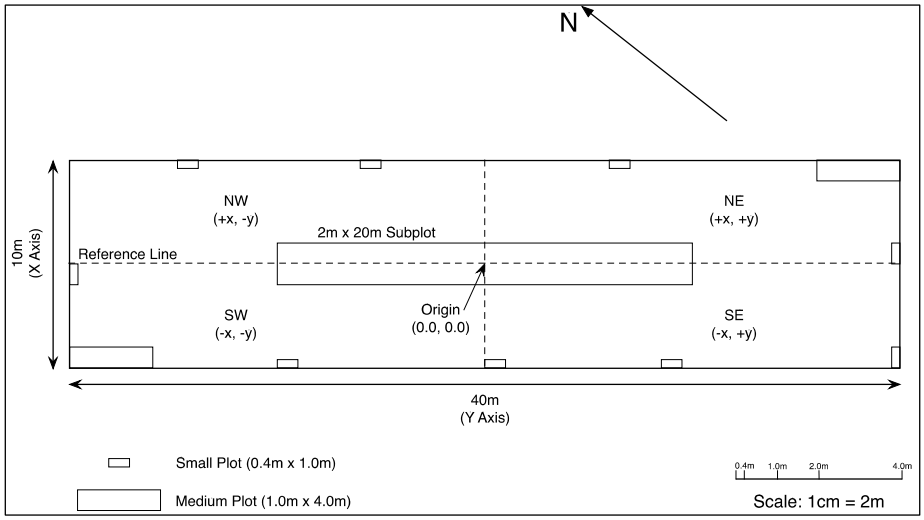
SUPPLEMENTARY MATERIALS SI. Vegetation plot configurations and survey information

Supplementary Materials SI – i. SK-FEC survey: Vegetation information was collected across a 10 m × 10 m square plot and reported as average percent cover by layer.



Supplementary Materials SI – ii. WBEA survey: Understorey vegetation information added in 2004. Large plots measuring 10 m × 40 m were established at each site. Vegetation information was collected across 10 small subplots (seen in Figure legend), 2 medium subplots (seen in Figure legend) and 1 large subplot in the centre, all within the large plot. Vegetation information was collected using the Daubenmire method of cover class estimation and reported as species composition percent.



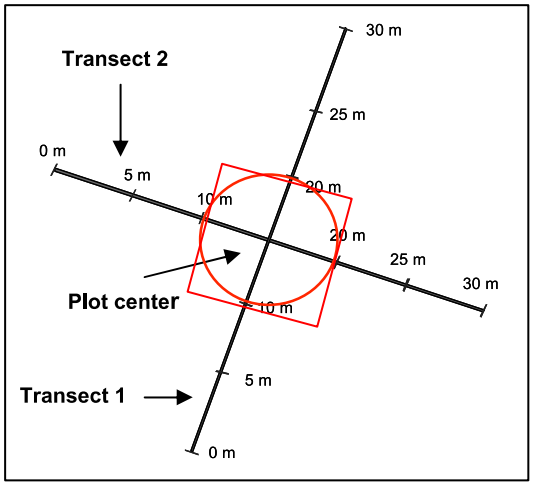
Source: Clair, T. A. and K. E. Percy (Editors) 2015. Assessing forest health in the Athabasca Oil Sands Region. *WBEA Technical Report*. 2015-05-25, 180 pp +Appendices.

Supplementary Materials SI – iii. Daubenmire Cover class estimation method

Daubenmire Cover Classes

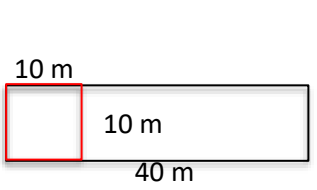
Cover Class	Canopy Coverage	Midpoint of Range
1	0 to 5%	2.5%
2	6 to 25%	15.0%
3	26 to 50%	37.5%
4	51 to 75%	62.5%
5	76 to 95%	85.0%
6	96 to 100%	97.5%

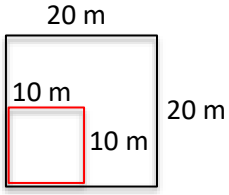
Supplementary Materials SI – iv. Vegetation sampling plot configuration used in the NFI survey. Vegetation information was collected across a 10 m × 10 m ecological plot at the centre of the site (seen by the square) and reported as percent cover by layer.

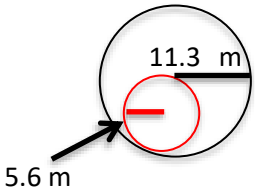


Source: Canadian Forest Inventory Committee (CFIC). (2008). Canada's National Forest Inventory: Ground Sampling Guidelines. *Natural Resources Canada*, Canadian Forest Service, Pacific Forestry Centre, Victoria, British Columbia. ISBN: 978-1-100-11330-2

Supplementary Materials SI – v. Vegetation sampling plot configuration used the NWT-FEC survey. Sampling began with a 20 m × 20 m plot where site characteristics and tree species and percent cover were recorded. Plot shapes were either rectangular (10 m × 40 m), square (20 m × 20 m) or circular (with radius of 11.3 m^2) to reduce topographic variability. Within each plot, smaller subplots (in red) were established to record shrub, herb, grass, moss and lichen species. Subplot shapes were either square (10 m × 10 m) or circular (radius of 5.6 m^2) depending on the site topography and vegetation information was reported as percent cover.







SUPPLEMENTARY MATERIALS SII. GradientForest Information

Supplementary Materials SII – i. Random forest and extended forest components, description and component use.

RANDOM FOREST & EXTENDED FOREST FUNCTION				
Component	**Goodness-of-fit values/Predictive Performance → R^2_S**	**Accuracy Importance Values**	**Raw Importance Values**	**Density of splits**
Description	R^2_S values given for a forest of any given species	Values are given for each predictor; determines how important a variable is for the model (predictive power)		Obtained in randomForest through Kernel density of estimations
Component Use	GradientForest: used in multiple plots; R^2_S values are partitioned into contributions (R^2_{SP}) from each predictor in proportion to the predictor importance	GradientForest: used in Conditional Importance Plot	GradientForest: used in Cumulative importance plots (both species and community)	GradientForest: used in densities plot

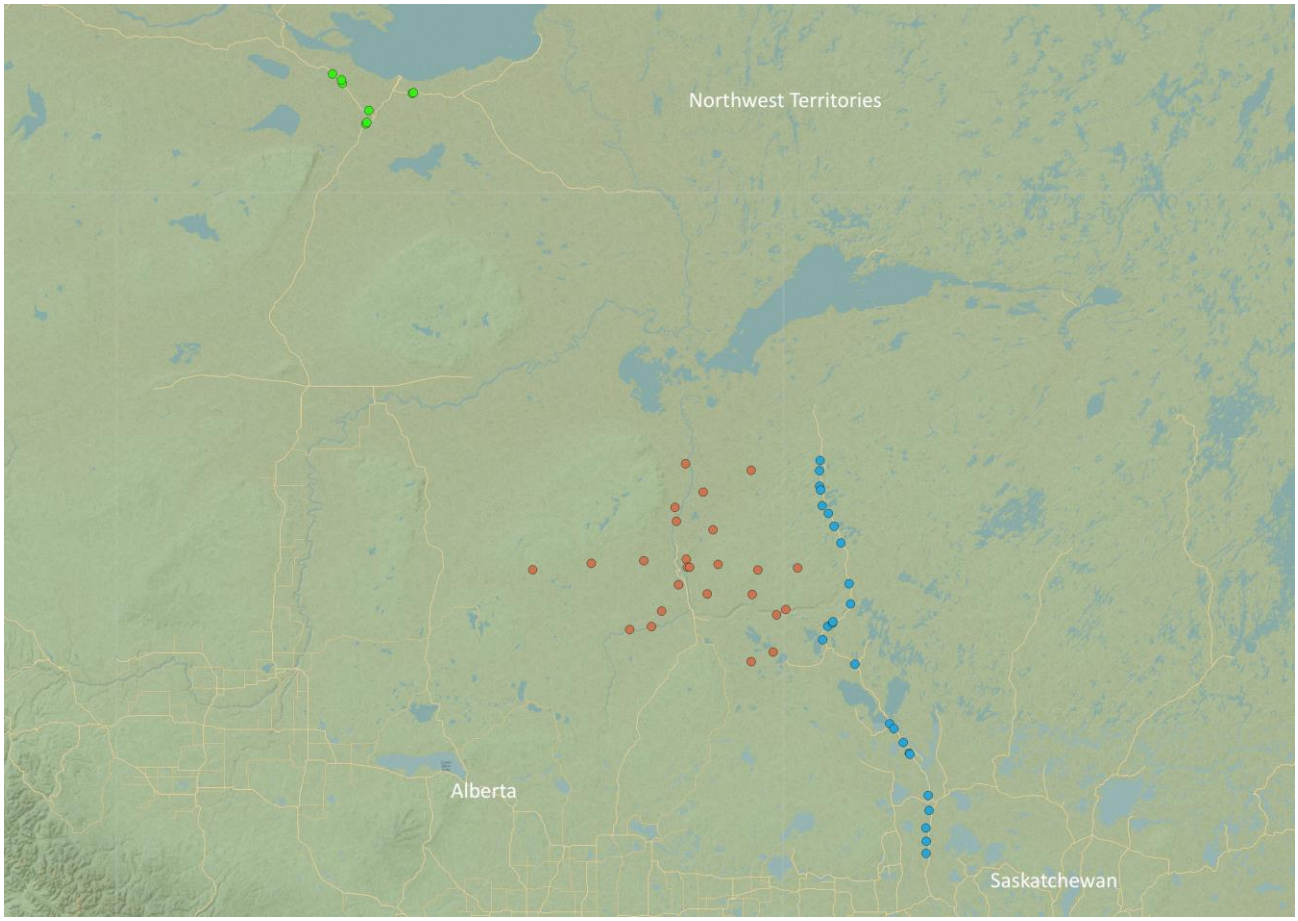
Supplementary Materials SII– ii. GradientForest outputs, components included, descriptions and additional information.

GRADIENT FOREST OUTPUT				
Plot Type	**Overall Conditional Importance Plot**	**Densities Plot**	**Cumulative Importance Plot (Species & Community)**	**Overall Performance (Species) R^2 Plot**
Included Components	• **Overall R^2 weighted importance** • **Accuracy importance**	• **Density of splits (black curve)** • **Density of data (red curve)** • **Ratio of densities (blue curve)** • **Ratio = 1 (dashed blue curve)** • **Binned Split Importance Values (grey bars)**	• **Individual species turnover functions** • **Community turnover functions**	• **R^2_{SP} values**
Output Description	Identifies the amount of variance in the model that is explained by each predictor and whether a predictor has predictive power	Identifies regions across an environmental variable of higher importance for plant species compositional change	Reveals Species associated with community thresholds	Shows species distributed according to their specific R^2 value; Indication of how well a species was predicted by all environmental variables
Additional Information	• **R^2_S are partitioned into contributions (R^2_{SP})** • **Sum of all R^2_{SP} = overall R^2 weighted importance (given to each predictor)**	• **Ratio of densities = $\frac{Density\ of\ splits}{Density\ of\ data}$** • **Area under the Curve = R^2 weighted importance** • **High peaks (where ratio >1) represent thresholds** • **Binned split importance represents both split locations and relative importance on the gradient**	• **Species turnover function: distribute R^2_S from all species among the predictors in proportion to the accuracy importance values and along the predictor gradients according to the density of raw importances** • **Community turnover functions: Aggregate the normalized cumulative distribution, scale by R^2 weighted importance and standardize by density of data and average across all species**	• **Overall Performance R^2 = Sum of all R^2_{SP} values per species** • **Output only shows positive response**

*The average of all overall R^2 weighted importance values = Mean R^2 weighted importance (how much of the overall model variation is explained by all predictors).

SUPPLEMENTARY MATERIALS SIII.

Supplementary Materials SIII – i. Location of all 58 sites initially considered before the TDN gradient was used to reduce site to 46. Study sites are colour coded based on their survey of origin, with orange representing WBEA survey sites (n = 25), green representing NWT-FEC survey sites (n = 9) in green and blue representing SK-FEC survey sites (n = 24).



Supplementary Materials SIII – ii. List of all 11 nitrogen species, all 4 sulphur species, TDN and TDS and their associated summary statistics across all 46 study sites. Data source is Gem-Mach (Makar *et al.*, 2018.

Range denotes the minimum and maximum, respectively. Variables denoted by * refer to variables that were selected for gradient forest analysis following variable reduction methods.

CODE	ENVIRONMENTAL VARIABLE (eq $ha^{-1}yr^{-1}$)	RANGE	MEDIAN	MEAN	STANDARD DEVIATION
*TDN	Total Deposited Nitrogen	37.21–597.01	136.70	176.45	131.60
DHN_3	Dry Hydrazoic Acid	8.57–83.02	36.69	39.34	19.72
DNH_3	Dry Ammonia	0.28–114.08	5.11	14.36	24.88
DNO_2	Dry Nitrogen Dioxide	2.74–309.59	17.02	47.43	70.21
DNO	Dry Nitrogen Oxide	0.0098–21.53	0.20	1.99	4.48
DPAN	Dry Peroxyacetylnitrate	3.78–17.24	9.90	10.16	3.70
DHNO	Dry Nitroxyl	0.029–1.77	0.17	0.34	0.42
DRN_3	Dry Organic Nitrate	1.67–6.33	3.89	4.09	1.26
PNO_3	Dry Particle Nitrate	0.040–4.30	1.92	1.98	1.04
PNH_4	Dry Particle Ammonium	2.31– 14.44	8.37	8.40	3.19
*WNO_3	Wet Nitrate	3.34–28.77	18.36	17.36	6.51
WNH_4	Wet Ammonium	9.66–63.31	31.65	31.01	10.91
*TDS	Total Deposited Sulphur	23.82–1182.20	53.55	72.75	95.21
DSO_2	Dry Sulphur Dioxide	1.71–391.85	7.75	18.41	42.12
PSO_4	Dry Particle Sulphate	2.32–15.34	6.23	6.58	2.31
HSO_3	Wet Hydrogen sulfite	3.94–844.44	13.62	22.42	54.84
*WSO_4	Wet Sulphate	11.41–57.67	24.79	25.34	6.58

Supplementary Materials SIII – iii. List of all 27 bioclimatic variables and their associated summary statistics. Note that codes beginning in 'bio' are sourced from Bioclim data and codes beginning in 'sg' are sourced from Seedgrow data (McKenney *et al.*, 2006)

CODE	ENVIRONMENTAL VARIABLE	RANGE	MEDIAN	MEAN	STANDARD DEVIATION
*LATITUDE	Latitude (°)	56.25–60.88	57.08	57.54	1.31
*LONGITUDE	Longitude (°)	-113.74–-108.89	-110.45	-110.68	1.20
*ELEVATION	Elevation (m)	231–622.52	460.34	425.50	108.37
*bio_12	Annual Precipitation (mm)	359–483	451.50	441.87	31.95
bio_13	Precipitation of Wettest Period (mm)	14–22	20	19.35	2.25
bio_14	Precipitation of Driest Period (mm)	0	0	0	0
bio_15	Precipitation of Seasonality (mm) (C of V)	46–62	55	54	3.94
bio_16	Precipitation of Wettest Quarter (mm)	149–226	209	201.5	20.68
bio_17	Precipitation of Driest Quarter (mm)	48–66	59.5	58.65	4.52
bio_18	Precipitation of Warmest Quarter (mm)	146–226	209	200.74	21.53
bio_19	Precipitation of Coldest Quarter (mm)	54–66	60	60.28	3.07
*sg_01	Julian day number at start of growing season	115–136	123.5	124.33	6.20
sg_02	Julian day number at end of growing season	279–290	288	286.20	3.29
sg_03	Number of days of growing Season	144–176	165.5	162.87	9.32
*sg_04	Total Precipitation for period 1 (mm)	50–70.7	63.60	63.07	5.46
sg_05	Total precipitation for period 2 (mm)	49.30–76.3	64.4	63.34	7.48
*sg_06	Total precipitation for period 3 (mm)	209.40–324.1	304	293.27	31.26
sg_07	Total precipitation for period 4 (mm)	160–257.6	236.45	229.92	26.10
sg_08	Gdd above Base Temperature for Period 1	0–1	0	0.24	0.43
*sg_09	Gdd above Base Temperature for Period 2	194–245	219	218.06	14.17
sg_10	Gdd above Base Temperature for Period 3	942–1317	1150.5	1128.22	107.85
sg_11	Gdd above Base Temperature for Period 4	704–1113	930.5	910.15	117.48
*sg_12	Annual Mean Temperature (°C)	-2.8–1.64	0.44	0.026	1.28
sg_13	Annual Minimum Temperature (°C)	-8.12–-3.96	-4.96	-5.38	1.22
sg_14	Annual Maximum Temperature (°C)	2.52–7.23	5.70	5.43	1.34
sg_15	Mean Temperature for Period 3 (°C)	11.75–12.89	12.34	12.33	0.32
sg_16	Temperature Range for Period 3 (°C)	22.99–25.67	24.18	24.32	0.70

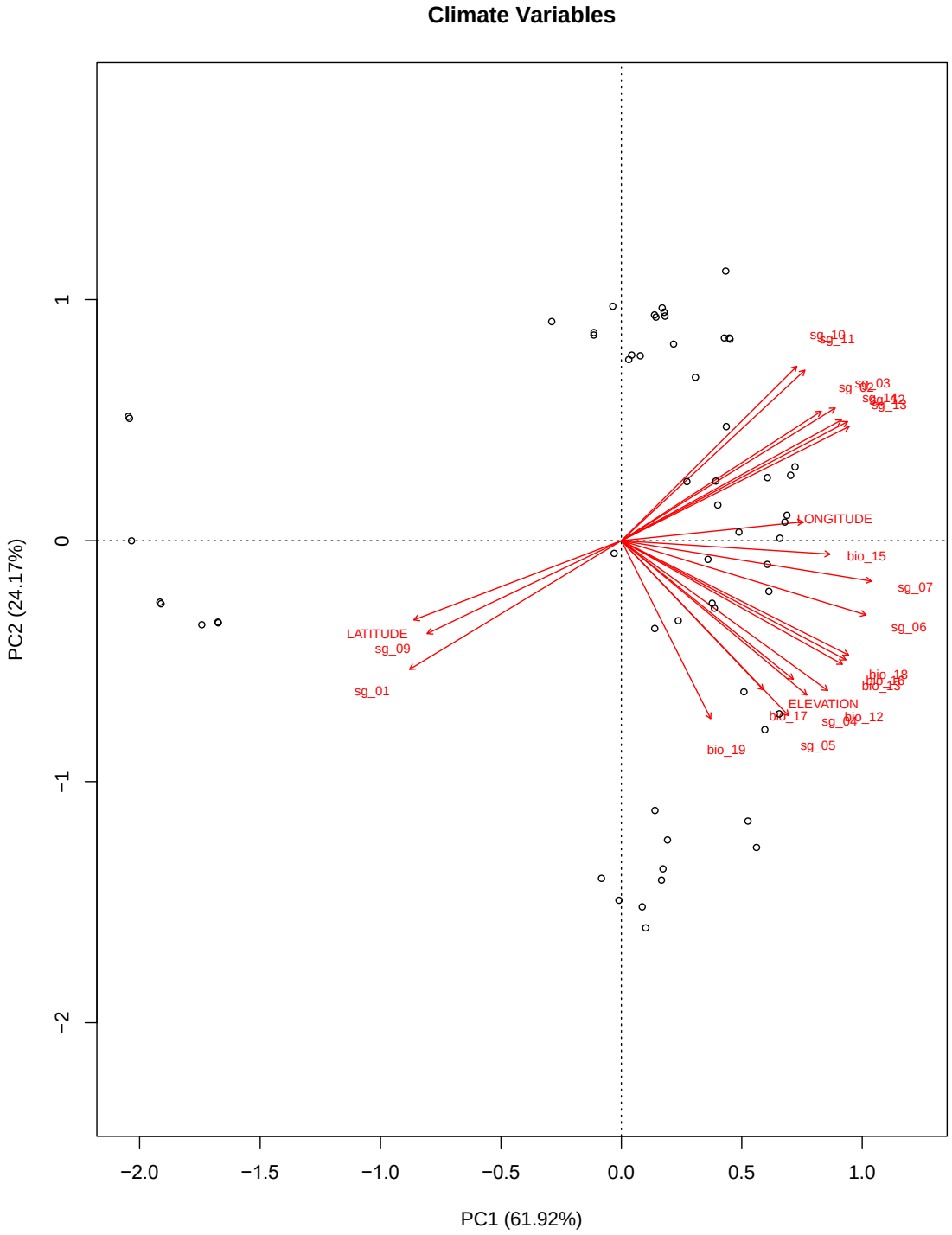
Range denotes the minimum and maximum, respectively. Variables denoted by * refer to variables that were selected for gradient forest analysis following variable reduction methods.

Supplementary Materials SIII – iv. Soil chemical variables (n = 40) of the 84 environmental variables initially analyzed.

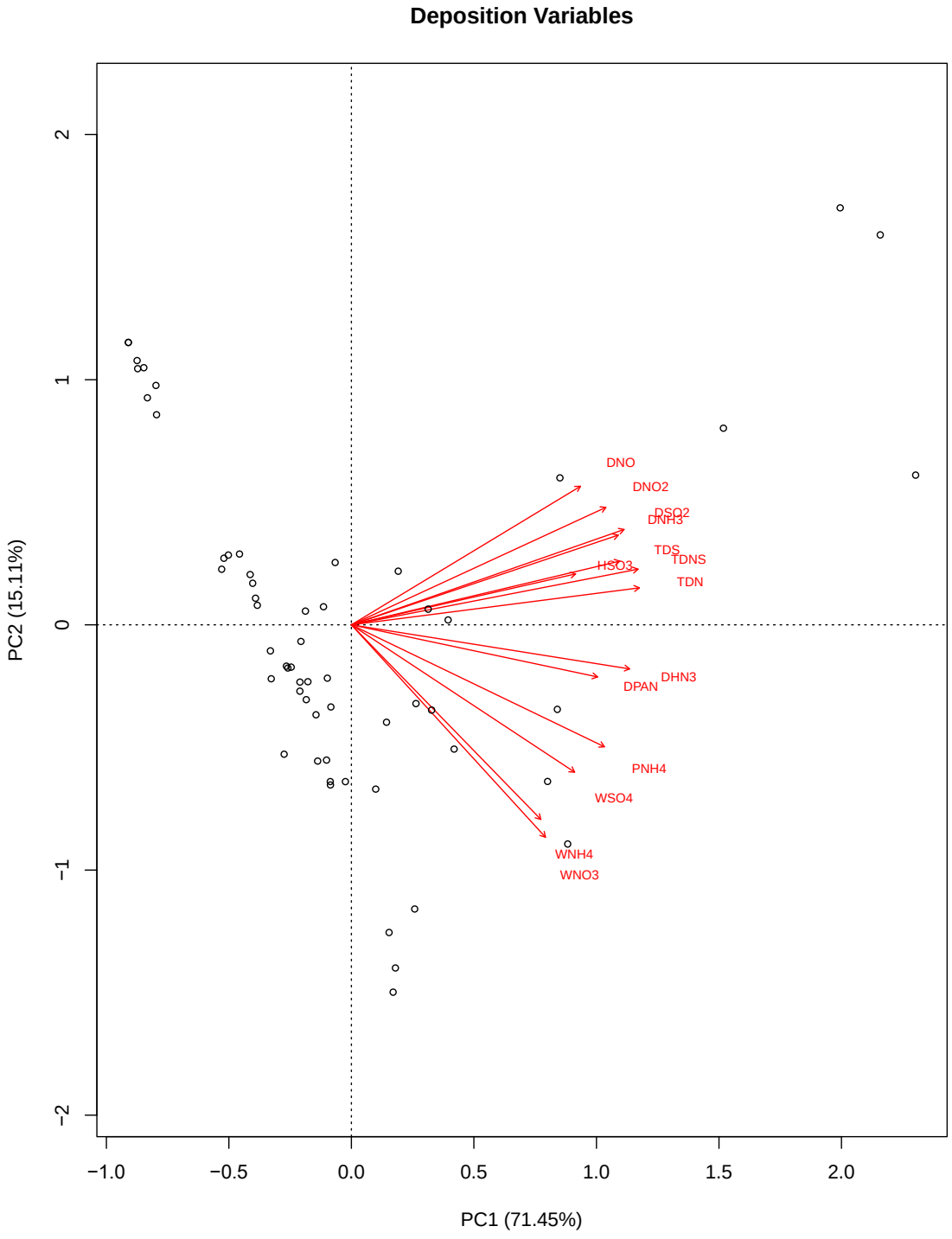
CODE	ENVIRONMENTAL VARIABLE	RANGE	MEDIAN	MEAN	STANDARD DEVIATION
*pH	pH – Soil	3.59–7.19	4.46	4.75	0.96
*LOI	Loss on Ignition – Soil	0.40–41.72	1.15	2.78	6.19
*X._Moisture	% Moisture – Soil	0.096–8.25	0.38	0.69	1.28
*Bulk_Density	Bulk Density – Soil	0–1.64	0	0.58	0.65
X._Porosity	% Porosity – Soil	0–64.47	0	23.82	26.55
Bulk_Density_ff	Bulk Density Fine Fraction – Soil	0–1.80	0	0.61	0.69
X._Porosity_ff	% Porosity Fine Fraction – Soil	0–57.97	0	22.46	25.10
*N_NH4	NH_4 N-Mineralization (µg/L) – Soil	0–11.65	2.86	4.09	3.20
Fl_H20	Fl Water Extractions (mg/L) – Soil	$0–8.76 \times 10^{-2}$	0	8.78×10^{-3}	1.94×10^{-2}
Cl_H20	Cl Water Extractions (mg/L) – Soil	0–0.35	0	4.65×10^{-2}	7.90×10^{-2}
NO2_H20	NO_2 Water Extractions (mg/L) – Soil	$0–4.33 \times 10^{-2}$	0	8.01×10^{-3}	1.10×10^{-2}
NO3_H20	NO_3 Water Extractions (mg/L) – Soil	0–0.60	0	1.80×10^{-2}	8.78×10^{-2}
*PO4_H20	PO_4 Water Extractions (mg/L) – Soil	0–0.46	0	3.81×10^{-2}	8.64×10^{-2}
SO4_H20	SO_4 Water Extractions (mg/L) – Soil	0–1.08	0	0.13	0.24
X._Clay	Particle Size Analysis- % Clay – Soil	0.26–8.94	1.01	2.26	2.06
X._Silt	Particle Size Analysis- % Silt – Soil	2.10–73.23	11.11	24.64	22.64
X._Sand	Particle Size Analysis- % Sand – Soil	20.78–97.64	87.04	72.97	24.42
X._Pebbles	Particle Size Analysis- % Pebbles – Soil	0–2.02	0	0.14	0.47
*Al_EC	Al Exchangeable Cations (mg/L) – Soil	0.03–2.43	0.23	0.44	0.48
Ca_EC	Ca Exchangeable Cations (mg/L) – Soil	0.28–54.81	1.06	5.22	12.44
*Fe_EC	Fe Exchangeable Cations (mg/L) – Soil	-8.80×10^{-2}–0.39	4.12×10^{-2}	8.23×10^{-2}	0.12
Mg_EC	Mg Exchangeable Cations (mg/L) – Soil	-1.09×10^{-3}–4.22	6.52×10^{-2}	0.31	0.75
*Mn_EC	Mn Exchangeable Cations (mg/L) – Soil	1.90×10^{-2}–0.54	0.15	0.17	0.12
*Na_EC	Na Exchangeable Cations (mg/L) – Soil	3.00×10^{-2}–0.70	0.10	0.14	0.12
K_EC	K Exchangeable Cations (mg/L) – Soil	5.00×10^{-2}–0.75	0.19	0.24	0.18
*X.N	CNS - % Nitrogen – Soil	5.33×10^{-3}–0.10	1.77×10^{-2}	2.49×10^{-2}	2.19×10^{-2}
X.C	CNS - % Carbon – Soil	0.21–3.50	0.48	0.65	0.61
*X.S	CNS - % Sulphur – Soil	$0–2.0 \times 10^{-2}$	2.81×10^{-3}	3.84×10^{-3}	4.28×10^{-3}
*C/N_Ratio	Carbon to Nitrogen Ratio – Soil	16.9–43.40	26.32	27.32	5.82
*pH_LFH	pH – LFH	3.02–7.01	3.96	4.23	0.90
*Fl_LFH_H20	Fl Water Extractions (mg/L) – LFH	0–0.98	0.14	0.20	0.21
*Cl_LFH_H20	Cl Water Extractions (mg/L) – LFH	0–10.32	0.79	1.34	1.80
*NO2_LFH_H20	NO_2 Water Extractions (mg/L) – LFH	$0–3.86 \times 10^{-2}$	1.05×10^{-2}	1.27×10^{-2}	1.04×10^{-2}
*NO3_LFH_H20	NO_3 Water Extractions (mg/L) – LFH	0–0.30	7.11×10^{-2}	7.67×10^{-2}	6.71×10^{-2}
*PO4_LFH_H20	PO_4 Water Extractions (mg/L) – LFH	0–11.52	4.36	4.22	3.84
SO4_LFH_H20	SO_4 Water Extractions (mg/L) – LFH	0–14.70	1.88	4.22	4.41
*X.N_LFH	CNS - % Nitrogen – LFH	0–1.48	0.99	0.95	0.28
X.C_LFH	CNS - % Carbon – LFH	0–48.64	38.49	36.44	9.84
*X.S_LFH	CNS - % Sulphur – LFH	0–0.22	6.37×10^{-2}	7.76×10^{-2}	4.25×10^{-2}
*C/N_Ratio_LFH	Carbon to Nitrogen Ratio – LFH	0–59.91	38.17	37.21	10.31

X. Represents %. Range denotes the minimum and maximum, respectively. Variables denoted by * refer to variables that were selected for gradient forest analysis following variable reduction methods.

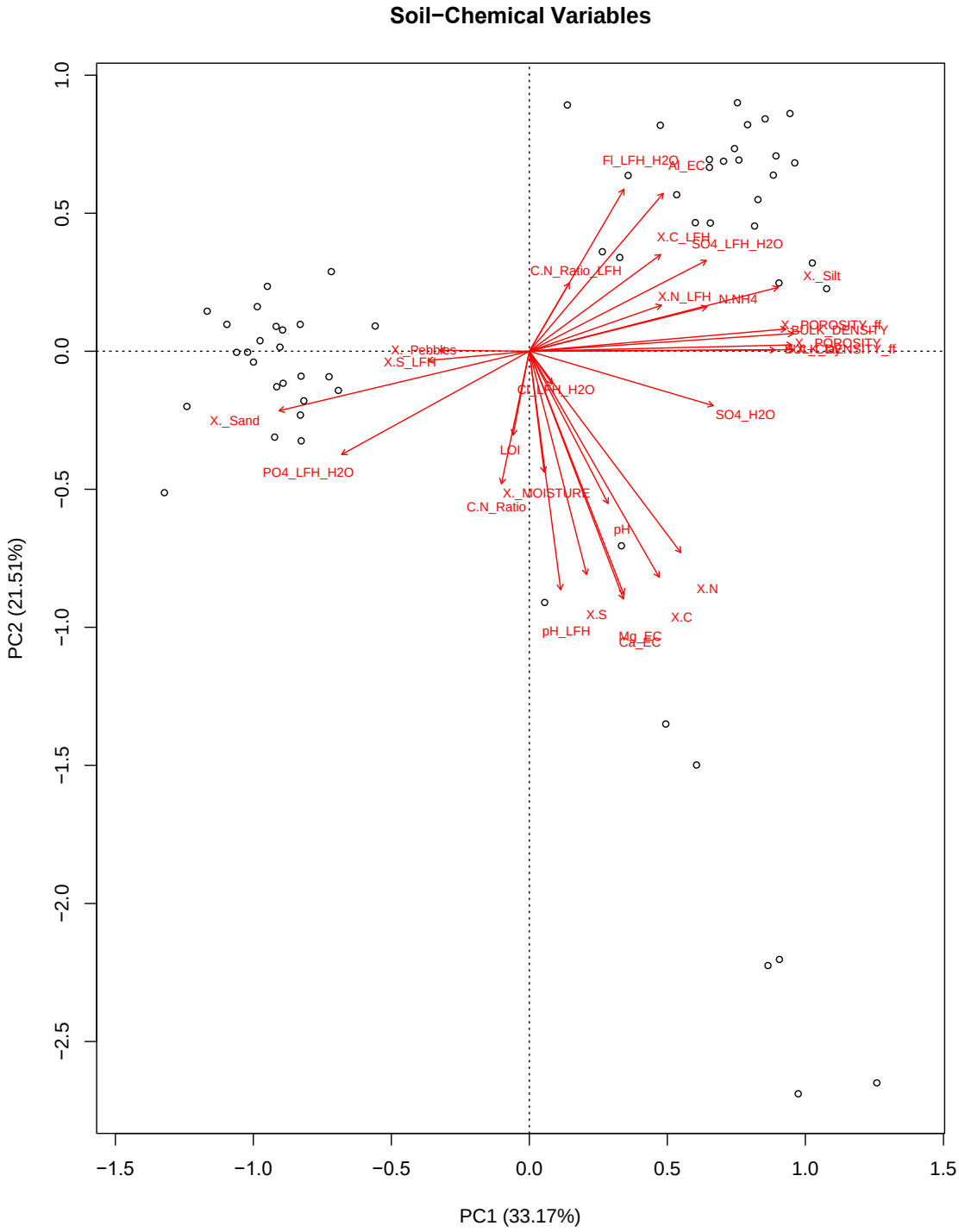
Supplementary Materials SIII – v. Ordination plot for Principal Component Analysis of climate variables, using the RDA function in vegan. Dots represent study sites.



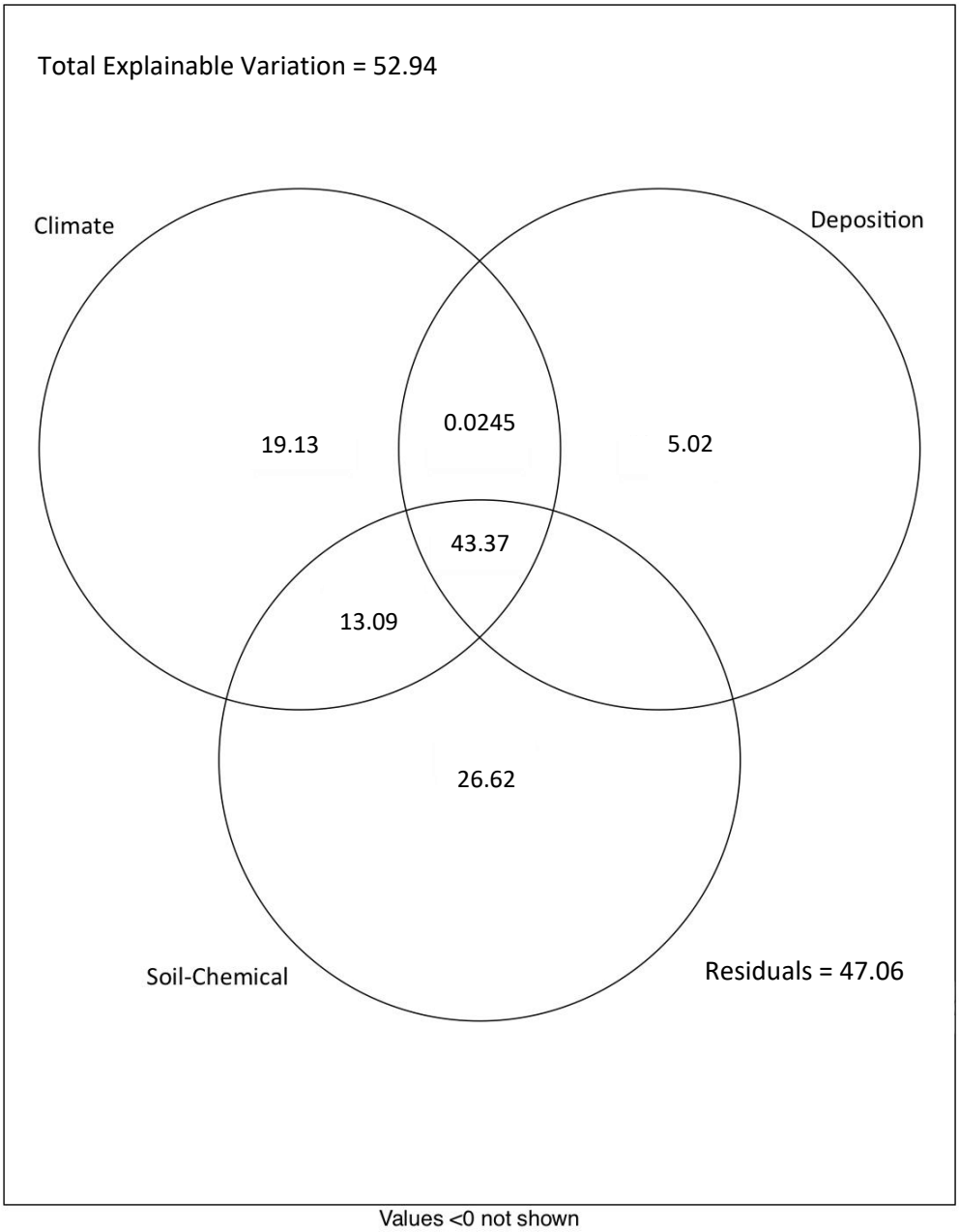
Supplementary Materials SIII – vi. Ordination plot for Principal Component Analysis of deposition variables, using the RDA function in vegan R package. Dots represent study sites.



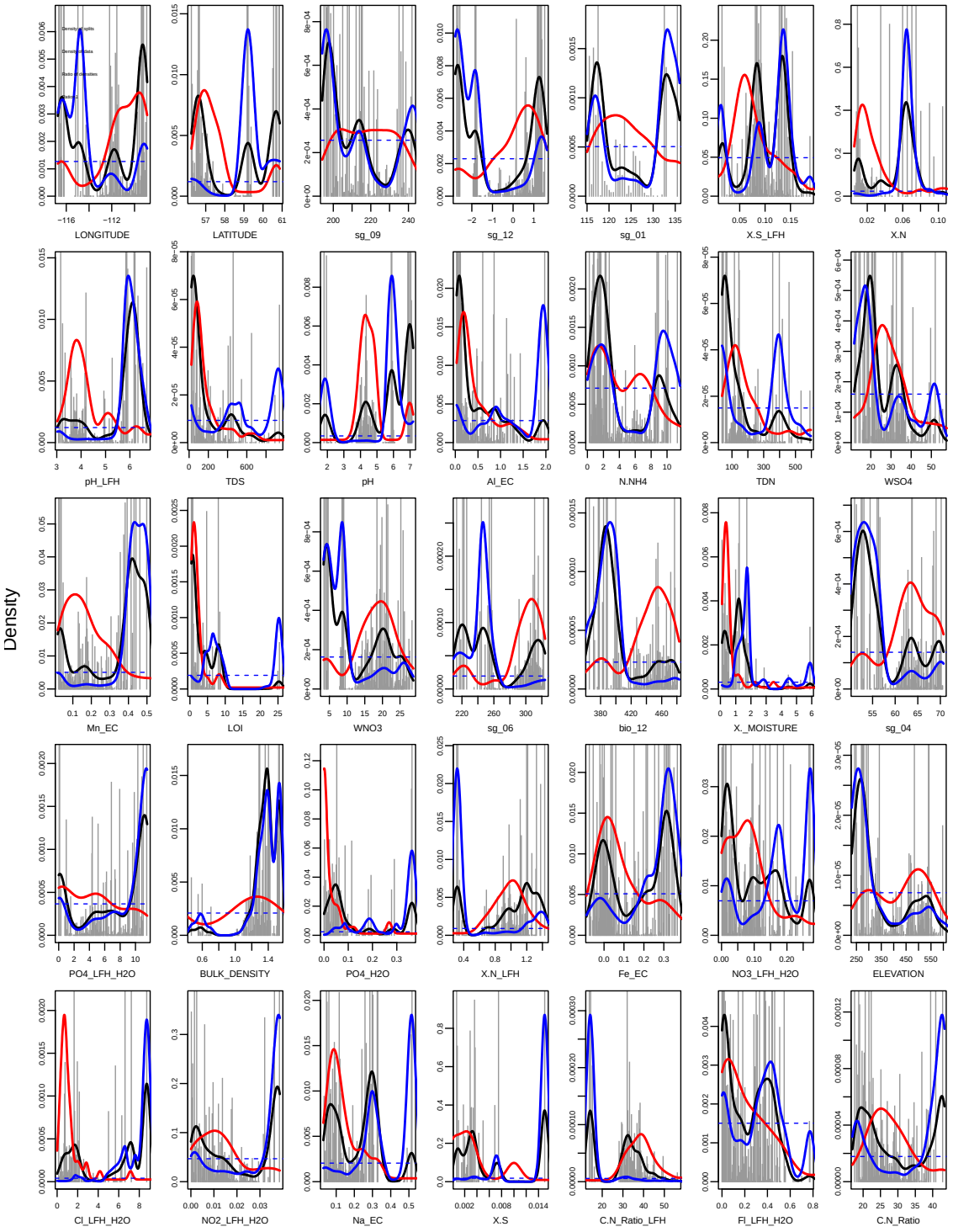
Supplementary Materials SIII – vii. Ordination plot for Principal Component Analysis of soil chemical variables, using the RDA function in vegan R package. Dots represent study sites.



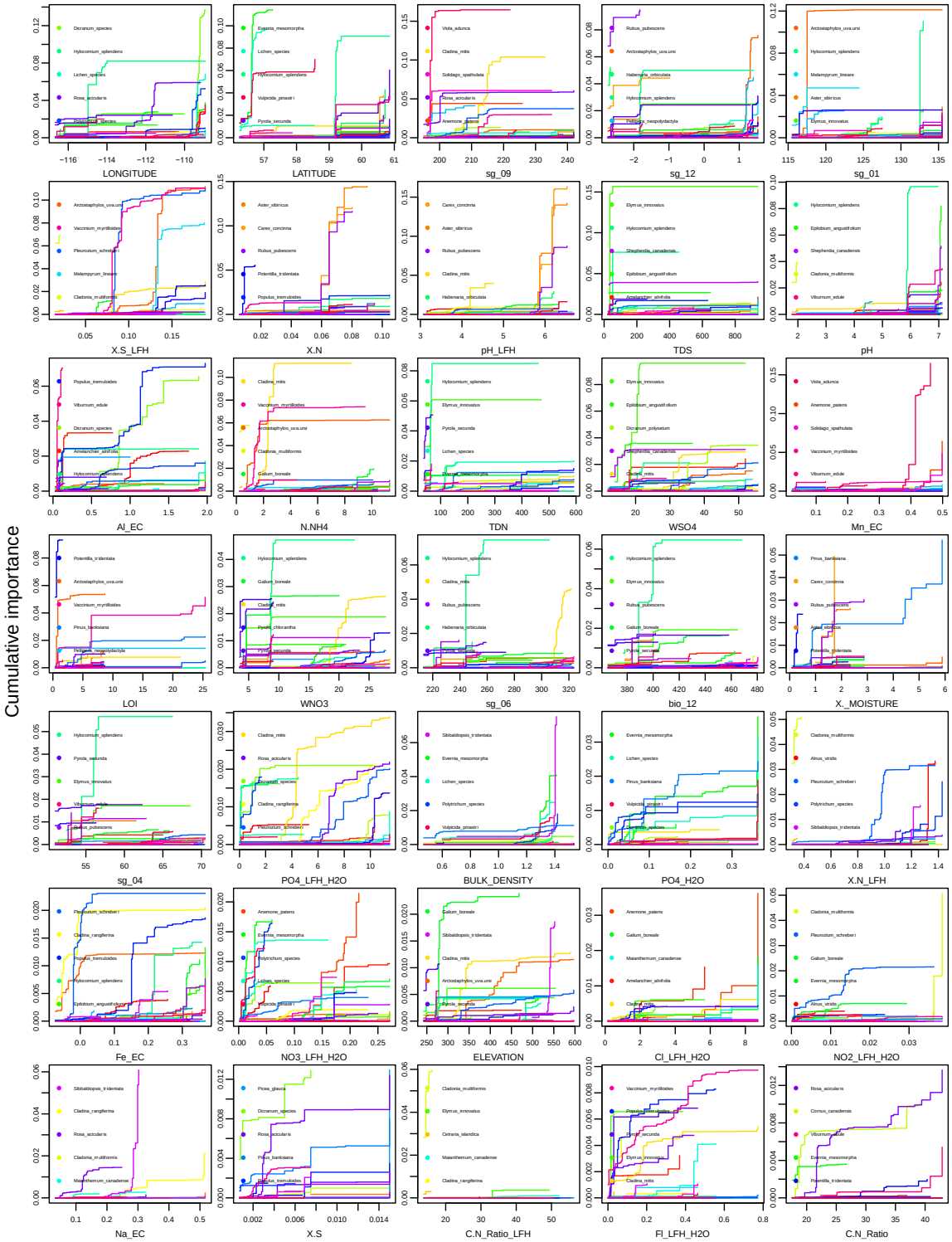
Supplementary Materials SIII – viii. Variation partitioning venn diagram for climate, deposition and soil-chemical variables and their joint-effects ($p < 0.001$). The joint-effect value between deposition and soil-chemical variables was -3.84 and is not show.



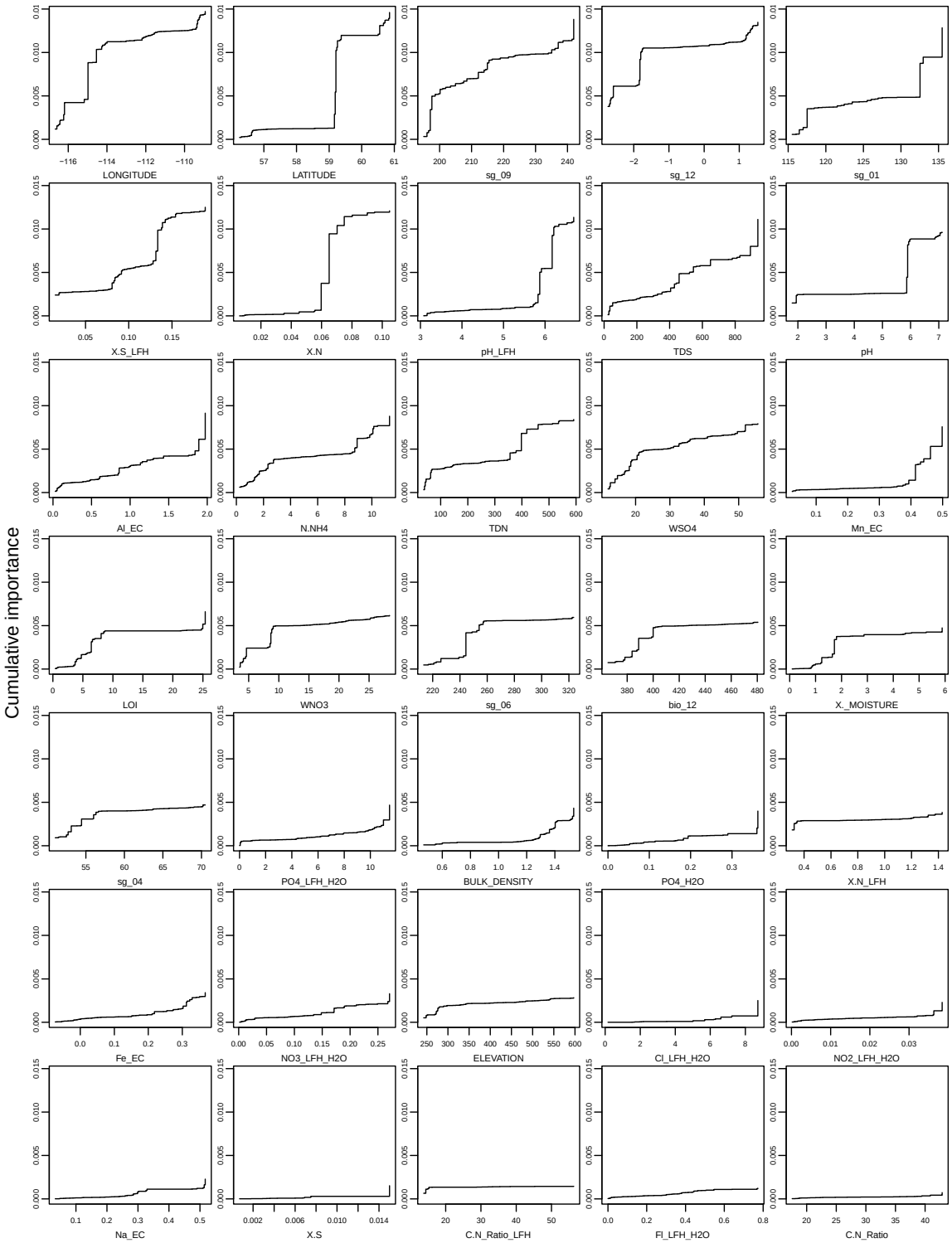
Supplementary Materials SIII – ix. GradientForest density plots for all remaining 35 environmental variables.



Supplementary Materials SIII – x. GradientForest species cumulative importance plots for all remaining 35 environmental variables. Only the top five responding species are displayed for each predictor.



Supplementary Materials SIII – xi. GradientForest community cumulative importance plots for all remaining 35 environmental variables.



Supplementary Materials SIII – xii. Correlations between biodiversity indices and all 35 variables selected for further analysis in gradientForest. Variables are divided into their 3 respective categories, Climate, Deposition and Soil-Chemical variables. *** p < 0.001, **p < 0.01, * p <0.05

		Pearson's Correlation Coefficient (r)		
Environmental Variable	**Variable Category**	**Species Richness**	**Shannon**	**Simpson**
Latitude	Climate	0.087	0.480 ***	0.369 *
Longitude	Climate	-0.458 **	-0.327 *	-0.230
Elevation	Climate	-0.276	-0.287	-0.210
Bio_12	Climate	-0.315 *	-0.436 **	-0.318 *
Sg_01	Climate	-0.199	0.478 ***	0.378 **
Sg_04	Climate	-0.353 *	-0.349 *	-0.220
Sg_06	Climate	-0.197	-0.532 ***	-0.412 **
Sg_09	Climate	-0.390 **	0.413 **	0.306 *
Sg_12	Climate	0.107	-0.506 ***	-0.405 **
TDS	Deposition	0.405 **	-0.157	-0.083
WSO_4	Deposition	0.284	-0.407 **	-0.298 *
TDN	Deposition	0.290	-0.216	-0.144
WNO_3	Deposition	0.096	-0.503 ***	-0.395 **
pH	Soil physico-chemical	-0.056	0.185	0.077
LOI	Soil physico-chemical	0.112	-0.017	-0.087
X._Moisture	Soil physico-chemical	0.126	0.047	-0.013
Bulk_Density	Soil physico-chemical	-0.169	0.608 ***	0.557 ***
$N.NH_4$	Soil physico-chemical	-0.222	0.486 ***	0.420 **
$PO_4\_H_2O$	Soil physico-chemical	0.042	0.345 *	0.285
Al_EC	Soil physico-chemical	-0.127	0.245	0.268
Fe_EC	Soil physico-chemical	0.095	0.669 ***	0.551 ***
Mn_EC	Soil physico-chemical	0.197	-0.362 *	-0.356 *
Na_EC	Soil physico-chemical	0.028	0.040	0.047
X.N	Soil physico-chemical	0.268	0.514 ***	0.423 **
X.S	Soil physico-chemical	0.405 **	0.459 **	0.305 *
C.N_Ratio	Soil physico-chemical	-0.006	-0.016	0.014
pH_LFH	Soil physico-chemical	0.106	0.205	0.093
$Fl\_LFH\_H_2O$	Soil physico-chemical	-0.297 *	-0.006	0.079
$Cl\_LFH\_H_2O$	Soil physico-chemical	0.379 **	0.180	0.152
$NO_2\_LFH\_H_2O$	Soil physico-chemical	0.038	-0.330 *	-0.382 **
$NO_3\_LFH\_H_2O$	Soil physico-chemical	0.335 *	0.122	0.065
$PO_4\_LFH\_H_2O$	Soil physico-chemical	0.510 ***	-0.215	-0.205
X.N_LFH	Soil physico-chemical	0.0075	0.295 *	0.297 *
X.S_LFH	Soil physico-chemical	0.340 *	-0.086	0.020
C.N_Ratio_LFH	Soil physico-chemical	-0.252	-0.054	0.039