Appendix 12 of BD5104
Heather nutrition

Plant nutrient content

The purpose of this Appendix is to further describe the methods and findings relating to the nutrient content of the *Calluna* plants grown under different management regimes which is described in Section 4.2.5.1 of the main body of the report for project BD5104. The method summaries, results and discussions are not repeated here but instead a more detailed description of the methods used to produce those results are given. Additionally, the maximum, minimum and average concentrations of each element for each site during the two time periods (**Table A12.1**) and the same values for each management in the two time periods (**Table A12.2**) are presented.

Collecting Calluna

The same *Calluna* plants as were cut for LAI, biomass and volume (see Appendix 3 and Section 4.2.5.2 in the main report) were analysed for plant nutrient content. Pre-management *Calluna* plants were cut from the 29 cm diameter circles used for net ecosystem exchange (NEE) measurements (see Appendix 6) before mowing and burning were carried out (March/April 2013). Post-management, the *Calluna* plants were cut from a patch the same size as the NEE circle where the *Calluna* cover was representative of that across the plot (18-20th August 2015).

Acid digests

The oven dried 'leafy' *Calluna* subsamples from the NEE plots were ground in a ball mill. Approximately 0.5 g of each ground subsample was weighed on a four point balance and placed in a Kjeldahl tube with 10 ml of 70% nitric acid (AnalaR NORMAPUR® grade, VWR International LLC, Radnor, PA, USA). A glass marble was placed on top of each tube and tubes were left overnight. For each block (18 tubes), there was a 'blank' consisting of only nitric acid (i.e. without the *Calluna* material). The following morning, the tubes were heated by increasing the temperature by 10°C every 15 minutes in a heating block until 60°C was reached. Tubes were left at 60°C for 3 hours then heated to 110°C using the same incremental method. After a further 6 hours, the tubes were removed from the heating blocks and left to cool overnight.

A small quantity (5-10 ml) of ultra-pure deionised water was added to each tube and swirled to mix. Each sample was separately filtered through a hardened ashless filter paper (125 mm diameter, No. 540, Whatman, GE Healthcare Life Sciences, Little Chalfont, UK) into a centrifuge tube. The marble and Kjeldahl tube were also rinsed into the filter paper and the filter paper was rinsed around with ultra-pure water. The samples were then re-filtered through clean filter papers into volumetric flasks and each sample made up to 100 ml with ultra-pure water. Samples were stored in clean centrifuge tubes at room temperature. Acid digests of the pre-management *Calluna* were carried out between 13th and 28th May 2015 in three separate batches and post-management *Calluna* samples were digested between 14th and 30th October 2015, also in three batches.

ICP elemental analysis

All samples were diluted by half to run in an inductively coupled plasma mass spectrometer (ICP; iCAP 7000 Series ICP spectrometer, Thermo Scientific, Waltham, MA, USA), with 10 ml of sample mixed with 10 ml of ultra-pure water in clean centrifuge tubes. Blanks and washes were both nitric acid, which was diluted to the same concentration as that in the samples, and two washes were run after every 12 samples. A certified reference material (Birch leaf Standard, CatNo. B2166, Elemental Microanalysis Ltd, Okehampton, UK) was also run between the two washes. Element concentrations were calibrated using 0.5, 1, 2, 5, 10 and 20 ppm concentrations of a multi-element standard (CertipurR, Merck KGaA, Darmstadt, Germany), containing K, Na, Ca, Mg, Fe, Al, Mn, Zn, Cu and Pb, which was made up in a nitric acid matrix. Phosphorus was similarly calibrated using 0.5, 1, 2, 5 and 10 ppm concentrations of a phosphorus standard containing H_3PO_4 (CertipurR, Merck KGaA, Darmstadt, Germany). Argon was used as the carrier gas. Pre-management samples were run in the ICP on 3rd June 2015 and post-management samples on 4th November 2015. Many of the Pb concentrations were below the machine limit of detection, resulting in negative concentrations being recorded. Therefore, Pb was excluded from further analyses.

C:N analysis

Approximately 50 mg of each ground oven dried 'leafy' *Calluna* subsample taken from the NEE plots was accurately weighed on a 4 point balance and folded into a tin foil cup (Art-No. 05 000 429, Elementar Analysensysteme GmbH, Hanau, Germany) to form a tight ball. The samples were analysed using the "Plant500" method in a C:N analyser (vario Macro, Elementar Analysensysteme GmbH, Hanau, Germany). Standards were glutamic acid and the results were factored according to the standards. For the pre-management *Calluna*, C:N analysis was carried out between 7th May and 18th June 2015. For the post-management *Calluna*, the C:N analysis was carried out between 9th November and 2nd December 2015.

Data analysis

Statistical analyses were carried out in R version 3.3.1 (R Core Team, 2016). Following Zuur *et al.* (2009), residuals were plotted against fitted values and visually assessed for normality and homogeneity of variance. Where a transformation was used on the data, residuals of the transformed data were assessed. The critical p-value chosen for significance was 0.05. All elemental concentrations were calibrated using the appropriate standards (phosphorus or multi-element) and converted from ppm to $\mu g\ g^{-1}$ dry material or percentage of dry material (whichever was most appropriate for the concentrations) using sample dry weight and dilution concentration. All values which appeared to be produced as a result of machine error (i.e. were negative or apparently completely devoid of a particular element) were removed. Additionally, one LB (mown with brash left) post-treatment sample from Nidderdale was missing and was therefore excluded from all analyses. This meant the number of replicates from each group was not the same for all elements.

Linear mixed models were implemented as described for species richness and diversity (see Appendix 3) with the following exceptions: the time periods, managements and sites were used as fixed effects in the mixed model. The only random effect used was the block the plots were in as there was only one set of measurements post-management.

Results

The nutrient content of *Calluna* plants on all plots at all sites was determined for 12 elements. Of these, 11 were present in large enough quantities to be properly measured in all samples and are detailed in **Table A12.1** and **Table A12.2** to facilitate interpretation of the results in Section 4.2.5.1 of the main body of the report and to allow comparison to other studies. Lead (Pb) concentrations were at or below the detection limit in many samples, resulting in many near-zero and negative values. Pb was therefore excluded from the summary tables and further analysis.

Table A12.1 The minimum (Min), maximum (Max) and average (Av) concentrations of each of the 11 elements measured in *Calluna* leaves for each site pre- and post-management. Site abbreviations are used where Nidd is a contraction of Nidderdale, Moss is a contraction of Mossdale and Whit is a contraction of Whitendale. Concentrations are given as percentages (%) for nitrogen (N), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg) and calcium (Ca), and as $\mu g\ g^{-1}$ of oven-dried leaf material for iron (Fe), aluminium (Al), manganese (Mn), zinc (Zn) and copper (Cu).

Site and period	Value	%						$\mu g\ g^{-1}$				
		N	P	K	Na	Mg	Ca	Fe	Al	Mn	Zn	Cu
Nidd pre	Min	1.02	0.05	0.12	0.03	0.10	0.18	121	91.5	70.1	28.2	8.71
	Max	1.53	0.15	0.22	0.09	0.22	0.59	214	134	383	106	14.8
	Av	1.23	0.09	0.16	0.05	0.16	0.40	160	108	214	49.8	12.4
Nidd post	Min	1.08	0.04	0.15	0.04	0.12	0.15	47.0	19.1	174	17.3	5.92
	Max	2.82	0.16	0.58	0.24	0.33	0.54	581	134	1753	125	15.3
	Av	1.84	0.10	0.40	0.12	0.25	0.42	155	56.1	899	59.1	12.1
Moss pre	Min	0.95	0.06	0.12	0.06	0.15	0.30	93.6	93.0	68.6	24.4	8.67
	Max	1.22	0.14	0.23	0.31	0.20	0.44	213	156	366	205	13.1
	Av	1.07	0.10	0.19	0.14	0.18	0.38	143	109	211	43.1	11.1
Moss post	Min	0.94	0.04	0.29	0.05	0.18	0.30	74.8	21.7	105	23.9	9.99
	Max	2.92	0.25	0.83	0.21	0.38	0.56	148	190	1900	62.2	15.8
	Av	1.81	0.12	0.46	0.12	0.26	0.40	102	44.1	1122	37.5	12.0
Whit pre	Min	1.18	0.06	0.13	0.04	0.11	0.18	97.2	91.9	54.6	28.2	10.3
	Max	1.75	0.17	0.28	0.13	0.20	0.42	424	249	571	75.2	17.5
	Av	1.41	0.11	0.19	0.08	0.14	0.27	197	133	204	42.1	13.9
Whit post	Min	1.34	0.06	0.24	0.04	0.17	0.26	88.7	34.7	125	30.9	9.73
	Max	2.76	0.17	0.66	0.20	0.35	0.59	356	186	1649	110	16.6
	Av	2.01	0.10	0.41	0.12	0.27	0.44	156	67.4	834	50.5	12.2

Table A12.2 The minimum (Min), maximum (Max) and average (Av) concentrations of each of the 11 elements measured in *Calluna* leaves for each management pre- and post-management. Management codes are used when DN represents unmanaged (uncut), BR represents mown plots with the brash removed, LB represents mown plots with the brash left and FI represents burnt. Concentrations are given as percentages (%) for nitrogen (N), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg) and calcium (Ca), and as µg g^{-1} of oven-dried leaf material for iron (Fe), aluminium (Al), manganese (Mn), zinc (Zn) and copper (Cu).

Mgmt and period	Value	%						µg g^{-1}				
		N	P	K	Na	Mg	Ca	Fe	Al	Mn	Zn	Cu
DN pre	Min	0.96	0.06	0.12	0.05	0.11	0.18	116	93.0	54.6	35.3	10.4
	Max	1.51	0.13	0.23	0.31	0.19	0.51	216	145	383	105	16.5
	Av	1.20	0.10	0.18	0.10	0.15	0.34	164	113	227	52.5	13.2
DN post	Min	0.94	0.04	0.24	0.05	0.18	0.33	87.8	35.8	105	30.6	10.1
	Max	1.81	0.10	0.49	0.13	0.26	0.52	344	186	639	110	14.9
	Av	1.33	0.07	0.33	0.08	0.21	0.40	152	81.4	290	50.7	11.7
BR pre	Min	1.02	0.05	0.12	0.03	0.10	0.19	113	95.3	68.6	24.9	8.71
	Max	1.65	0.13	0.24	0.19	0.19	0.51	266	165	571	74.9	16.0
	Av	1.25	0.09	0.18	0.09	0.16	0.34	164	115	215	41.4	11.8
BR post	Min	1.43	0.04	0.15	0.04	0.12	0.15	47.0	19.1	272	17.3	5.92
	Max	2.92	0.15	0.68	0.20	0.32	0.53	356	190	1753	78.9	16.6
	Av	1.98	0.11	0.41	0.12	0.26	0.42	121	54.3	1059	44.2	12.0
LB pre	Min	0.95	0.06	0.13	0.03	0.10	0.18	113	91.5	98.9	24.4	10.0
	Max	1.75	0.15	0.24	0.22	0.22	0.59	424	249	549	50.2	17.5
	Av	1.24	0.10	0.18	0.09	0.16	0.36	183	123	205	39.2	13.3
LB post	Min	1.41	0.07	0.27	0.05	0.17	0.26	74.8	21.7	450	32.0	9.47
	Max	2.55	0.17	0.57	0.21	0.35	0.54	341	118	1900	114	15.8
	Av	1.93	0.12	0.42	0.13	0.27	0.40	132	48.8	1047	44.5	12.2
FI pre	Min	1.04	0.06	0.12	0.05	0.15	0.23	93.6	91.9	78.9	28.2	8.67
	Max	1.52	0.17	0.28	0.13	0.20	0.51	213	156	253	99.7	13.3
	Av	1.21	0.11	0.20	0.08	0.18	0.36	143	110	189	43.2	11.5
FI post	Min	1.62	0.09	0.35	0.06	0.23	0.37	88.7	25.3	644	32.3	10.1
	Max	2.76	0.17	0.83	0.24	0.38	0.59	197	81.8	1875	125	15.0
	Av	2.16	0.13	0.55	0.13	0.29	0.49	128	47.5	1218	64.5	12.7

References

R Core Team (2016) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.