**Appendix 9 of BD5104**

**Micro-topography and peat shrinkage/expansion assessments**

The purpose of this Appendix is to further describe the methods relating to micro-topography and peat shrinkage and expansion assessments which are described in Sections 4.2.3 and 4.4.1, respectively, of the main body of the report. The method summaries, results and discussions are not repeated here but instead the full details of the methods are given.

## Methods

### Micro-topography

The variation in the peat surface was assessed on all 72 monitoring plots across the three sites in September 2015, at Nidderdale on 14$^{th}$, Mossdale on 15$^{th}$ and Whitendale on 16$^{th}$.

Two 60 cm canes were marked 20 cm from the bottom and notched 10 cm from the top. The canes were inserted into the peat at a right angle to the peat surface, exactly to the 20 cm mark, on either side of a 5 x 5 m plot. A piece of twine was tied tightly between the two canes so that it sat within the notches and was exactly 30 cm high either side of the plot. The offset between peat surface and the twine was measured at nine marked points (every 50 cm) along the 5 m section of twine spanning across the 5 x 5 m plot using a 1 m long wooden ruler. Transects spanned the plots (i.e. was across the slope as opposed to down it) and there were five transects per plot, with the first being 50 cm into the plot. This provided 45 points across each of the 5 x 5 m plots.

The mean offset and the standard deviation of the offsets were calculated for each plot and both were tested with a two-way ANOVA (management and site as factors).

### Peat shrinkage and expansion

Peat water tables and peat moisture can change considerable throughout the year due to environmental (e.g. prolonged summer dry period) but also vegetation (e.g. different evaporation rates) conditions. As changes in water content can affect the bulk density of the peat, the peat surface is likely to fluctuate between seasons and vegetation type. Importantly, such shrinkage and expansion rates (commonly referred to as 'Mooratmung') can then affect carbon stock assessments when a) assuming constant bulk densities, or b) only sampling a shallow area (i.e. not the entire peat column). The following section outlines two assessments in this respect, one was a controlled incubation study (in the greenhouse) the other one a field study (at all three project sites).

### Greenhouse cores

Peat cores of 1 m length were obtained on 20$^{th}$ November 2013 from a flat area of deep peat at Mossdale using a 5 x 5 cm box corer. In total, 24 cores were removed; eight each from areas dominated by *Sphagnum* species, *Eriophorum* species and *Calluna vulgaris*. Cores were cut into 15-20 cm sections to avoid excess compression on removal from the auger. Each core was reassembled in a 1 m long, 5 x 5 cm square uPVC ducting pipe which had a detachable side cover. Care was taken to place the deepest part of the core at the end of the ducting pipe to minimise slippage when the pipe was stood upright. Further, a uPVC end cap was added to the end of each pipe, once the detachable side had been replaced, to minimise slippage and peat loss at the bottom of the tube. The end cap was not sealed on in order to enable drainage and water movement throughout the cores.

Cores were transported to an unheated greenhouse and stood upright in a minimum of 30 cm of water. On 25th November 2013, the ducting pipes containing the cores were individually and randomly placed into larger tubes consisting of a 95 cm long, 11 cm diameter uPVC tube (Plumb Center, Wolseley UK Ltd, Leamington Spa, UK) glued into an indentation on a 12 cm square of PVC (Biology Workshops, University of York, UK) using Bostik All Purpose Extra Strong Glue (Bostik Ltd, Stafford, UK). Tube bases were sealed with the glue to make them watertight. A 1 cm diameter hole was drilled through each tube 15 cm from the top. Half of the tubes had an additional hole drilled through them 35 cm from the top, into which a watertight bung was fitted. All tubes were filled with water up to the 15 cm hole.

On 11th December 2013, four evenly-spaced 0.6 mm diameter holes were drilled along the detachable side of each pipe to aid drainage and water movement. Additionally, the top 4-5 cm of each core was sliced off to remove the surface vegetation and dense root layer and to provide a definitive peat surface. As this lowered the peat surface relative to the holes in the outer tubes, the removal of the top of each core raised the WTD to -15 cm. Four cores had resettled such that there was a gap between the bottom of the core and the end cap. A small piece (<1.5 cm) which had been removed from the top of these cores was added to the bottom to prevent sudden slippage later which may have impacted the measurements. The distance from the centre of the peat surface (i.e. where the vegetation was removed) to the top of the ducting tube was measured to the nearest millimetre and subtracted from the height of the ducting pipe. This was taken to be the starting height of each peat core.

On 22nd January 2014, the cores were assigned to one of four blocks, such that each block contained two *Calluna*- (C), two *Eriophorum*- (E) and two *Sphagnum*- (S) topped (i.e. cut off the first few centimetres of the previous vegetation) cores. Cores were arranged within blocks according to a Latin square, with one core of each species in a tube with two holes and one in a tube with one hole. A temperature logger (Tinytag Plus 2 – TGP-4017 data logger, Gemini Data Loggers Ltd, Chichester, UK) was fixed 30 cm from the bottom of one tube in each block. The loggers were on the corner tubes of the complete stand and fixed on the side of the block-facing side to protect them from direct sunlight. On the same four tubes, an additional temperature logger was attached at the top. These loggers had two probes which were placed inside the tube, one with its tip at -15 cm (i.e. with just the tip in the water) and the other with its tip at -30 cm (i.e. with the probe fully submerged), to monitor the water temperature.

Peat measurements commenced with temperature monitoring. The distance from the peat surface to the top of the ducting pipe was measured approximately 0.5 cm from each of the four corners of each core using a pair of callipers (Traceable Digital Carbon Fiber Calipers, Fisher Scientific, Pittsburgh, PA; accuracy ±0.2 mm) and subtracted from the height of the pipe (1 m) to obtain the height of the peat core. Water was added to tubes at least weekly so that the WTD never dropped more than 2 cm below the desired level. All cores were measured six times over the four months the WTD was maintained at -20 cm. This was Time Period (TP) 1.

On 17th April 2014, bungs were rearranged such that the WTD of the single-holed tubes (Set1) was raised to -5 cm and that of the two-holed tubes (Set2) was lowered to -40 cm. Due to the top of all cores being below the top of the piping tubes, the piping tubes being 5 cm taller than the outer tubes and the surface fluctuations of the cores themselves, these WTDs are the lowest that the peat cores experienced. Therefore, the WTDs experienced by the cores were between -5 cm and 0 cm for the wetter tubes and -40 cm and -35 cm for the drier tubes. Cores were measured by the same method 11 times over nine months, with intervals between measurements ranging from one to 11 weeks. This was TP2.

To test whether cores which had been under a specific WTD long-term would behave similarly when placed under another WTD, cores were swapped pairwise by species within blocks (e.g. both *Calluna*-topped cores within block

1 were swapped so that the WTDs were the opposite of that which they had been) on 21st January 2015. Cores were measured seven times over five months as previously. This was TP3.

On 24th June 2015, all bungs were replaced and the WTD was raised to -5 cm for all tubes to investigate whether all cores would rehydrate (i.e. expand) fully. Cores were measured six times over three months. This was TP4. On 2nd October, all tubes were fully drained to simulate a WTD of -100cm and left for a month for the peat to drain. After a month, the cores were measured twice in the following month. This was TP5.

**Greenhouse core bulk densities**

As well as the WTDs of the cores being switched on 21st January 2015, the top 4-5 cm of all cores from three blocks were removed with a sharp knife. These removed cuboids were measured in all three dimensions with callipers and oven-dried at 105°C in foil dishes until a constant weight was achieved. The bulk density was calculated by dividing the final dry weight by the volume, derived from the three side measurements.

**Field poles (rods)**

To verify the peat surface fluctuations of the greenhouse cores, 12 mm solid steel poles were installed in the field. All poles were at least 40 cm longer than the peat depth at their respective installation locations. A 1-2 mm groove was marked 20 cm from the top of each pole around the circumference. Poles were pushed vertically (at right angle to the peat surface) into the peat until they reached the bedrock and then hammered about 20 cm into the underlying bedrock with an iron mallet to prevent movement of the pole from frost heave or animal collision.

A custom-made coloured acrylic disc of 5 cm diameter with an internal circle of 1.5 cm (Biology Workshops, University of York, UK) was placed over each pole (to protect the peat surface). The vegetation beneath each disc was cleared away such that the discs could lie flat on the peat surface. The discs also provided a solid reference point of the peat surface for each measurement, even when vegetation grew back.

A pole was installed on each permanent plot, within 50 cm of a WTD meter, at each of the three sites. These poles were inserted at Nidderdale and Mossdale on 4th August 2014 and at Whitendale on 5th August 2014. A further 90 poles were installed at Mossdale in clusters of three on 5th and 7th August 2014. Within a group, poles were between 50 cm and 1 m apart. In the centre of each cluster, a 1 m long core which was 5 x 5 cm square was removed. This hole was used as a dipwell to measure the WTD. Six clusters of three poles were installed on areas dominated by *Sphagnum* species (i.e. the vegetation all three poles were in was over 70% *Sphagnum*), six on areas dominated by *Eriophorum*, six on mature *Calluna*, six on recently burnt *Calluna* and six on recently mown *Calluna*. For each of these species/management groups, three clusters were located on a flat slope (≤ 4°) and three on a steeper slope (> 5°).

Peat depth of all locations was measured and recorded prior to pole installation (using extendable drainage rods). The distance between the top of the pole and the disc on the peat surface was measured when poles were installed and a further seven times over the following two years. At the same time, the WTD was manually measured both on the plots and in the central holes of the groups of three. The distance from the top of the pole to the 20 cm groove was also measured to check whether the pole itself had expanded or contracted due to temperature.

**Data analysis**

All statistical analyses were carried out in R version 3.3.1 (R Core Team, 2016). The critical p-value chosen for significance was 0.05.

**Greenhouse cores**

The four measured peat heights for each core on each measurement occasion were averaged. The starting height of each core was subtracted from these peat heights to obtain the change in height for each measurement occasion. A three-way ANOVA was used to determine whether the change in peat height differed between PFTs, Sets (and thereby by WTD) and TPs. Only the final set of measurements within each TP was used as these represented the maximum change in peat height for each core within each TP. Where significant differences were detected, the "TukeyHSD" function was used to determine between which groups significant differences occurred.

**Greenhouse core bulk densities**

A two-way ANOVA, employing the "aov" function in the "stats" package (R Core Team, 2016), was used to investigate the effects of PFT (plant functional type) and WTD on the bulk density. Where significant differences were detected, the "TukeyHSD" function was used to determine between which groups significant differences occurred. A Shapiro-Wilk test ("shapiro.test" from the "stats" package; R Core Team, 2016) was used to test whether the residuals followed a normal distribution and Levene's test ("leveneTest" from the "car" package; Fox & Weisberg, 2011) was used to assess homogeneity of variance.

**Field poles**

Change in peat depth was calculated as the total pole length minus the distance from the disc on the peat surface minus the length of pole in the bedrock (initially calculated based on the pole length, starting peat depth and starting length of pole protruding from the peat). Similarly, change in WTD was calculated by subtracting the WTD on each measurement date from the WTD when the poles were installed.

The poles which had been installed on the permanent plots across the three sites ("plot data") were analysed separately from the additional 90 poles installed at Mossdale ("+90 data"). Linear mixed effects models employing the "lmer" function from the "lmerTest" package (Kuznetsova *et al.*, 2016) were used to test which factors affected the change in peat height. For the plot data, management, site and change in WTD were used as fixed effects, as were the interactions between them, and a random intercept was included with a nested structure of blocks in sites (to account for spatial heterogeneity) in measurement dates (to account for repeated measurements). For the +90 data, PFT, slope, change in WTD and the interaction between them were used as fixed effects, with pole cluster nested in measurement date as the random intercept.

Following the 10-step protocol in section 5.10 of Zuur *et al.* (2009), variables were dropped stepwise from each linear mixed effects model and the log-likelihood ratio and AIC value were used to assess whether a variable should be dropped or kept in the model. For the final models, the "Satterthwaite" option was used to calculate the denominator degrees of freedom as the missing heights of three poles during one measurement date for the +90 data, and the number of plots under each management within the plot data, resulted in an unbalanced design (Spilke *et al.*, 2005). Where significant interactions were found, the "glht" function with the "Tukey" option from the "multcomp" package (Hothorn *et al.*, 2008) was used to compare groups within the interaction terms.

**References**

Fox J, Weisberg S (2011) *An R Companion to Applied Regression, Second Edition*. Sage, Thousand Oaks, CA.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal*, **50**, 346–363.

Kuznetsova A, Brockhoff PB, Christensen RHB (2016) *lmerTest: Tests in Linear Mixed Effects Models*.

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

Spilke J, Piepho H-P, Hu X (2005) A Simulation Study on Tests of Hypotheses and Confidence Intervals for Fixed Effects in Mixed Models for Blocked Experiments with Missing Data. *Journal of Agricultural, Biological, and Environmental Statistics*, **10**, 374–389.

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