APPENDIX 5: pH, moisture and aeration of soils

A5.1 pH measurements

pH measurements were taken from fresh soil core samples in the field in July 2002. A 0.5 m auger (20 mm in diameter) was used to extract four soil cores on representative ground adjacent to each plot. The amount of compaction was estimated by subtracting the depth of the core hole from the actual length of the soil core in the auger. This compaction factor was then taken into account when taking a soil sample from the core between the 0.15 m to 0.20 m mark. Each sample was bagged and stored in a cool box to await pH measurements which were carried out within 6 hours of sampling. Two 10 ml scoops of fresh soil from each sample was placed in a beaker with 50 ml of distilled water (1:2.5) (Rowell, 1994). The suspension was mixed over a 15 minute period and then swirled over a glass gel electrode and temperature probe (HI 9024C, Hanna Ltd) recording the pH after 30 seconds. Before readings commenced, the electrode was left in buffer solution to ensure adequate wetting of the electrode bulb and then calibrated with pH 4 and pH 7 buffers.

A5.2 Volumetric soil moisture content

Volumetric soil moisture content was measured using a thetaprobe soil moisture sensor (Type ML2x, Delta-T Devices Ltd, Cambridge) in the field in August of each year. It was assumed that any water limitation to seedlings would occur in the warmer drier months of the growing season. After 2-3 days with no rain, four soil moisture readings were taken within each treatment plot. Ideally, the thetaprobe readings should have been taken when all soils were at field capacity, to standardise the procedure. However, this was impossible due to different soil types. Two readings were taken in columns 2 and 4 of each Latin Square with the thetaprobe placed left of each randomised *I. aquifolium* seedling. A soil specific calibration of the thetaprobe was carried out by following instructions outlined in the user manual (Delta-T Devices Ltd). Soil samples for calibration were collected from representative soil types under all three stand treatments.

A5.3 Soil aeration

Aerobic soil depth was measured using mild steel welding rods (Armstrong *et al*., 1976; Bridgham *et al*., 1991). This technique exploits the redox attributes of iron, which forms reddish-brown rust in the oxidised Fe^{3+} state but is reduced under

anaerobic conditions to grey Fe^{2+}. The boundary between these two conditions is approximated by the water table (Bridgham *et al*., 1991). Strong correlations have been found between the water table depth and the steel rod rusting depth for different soil types under different vegetation types (Carnell and Anderson, 1986; Bridgham *et al*., 1991). However, under fluctuating hydrology, a rapid drop in the water table results in the rod oxidation demonstrating a lag period and once heavy rust forms on the rods it does not dissolve upon reflooding (Bridgham *et al*., 1991).

Ideally frequent sequential sampling every month throughout the year would provide information on fluctuating hydrology and also capture readings over the period of higher water table. However due to the lack of time and logistics, one reading in the Autumn had to suffice. Rods were inserted into the soil in mid September and removed in mid November with a total incubation period of 68 days. Before insertion, rods were scrubbed clean to remove all traces of brown rust and patchy grey areas. The rods (6 mm diameter, 0.8m length) were pushed vertically into the soil adjacent to each plot on representative ground. Insertion occurred to a maximum depth of 0.5 m.

At the end of the sampling period, the position of the soil surface (horizon between humus and peat) was marked on each rod, before being removed, cleaned and dried. The soil surface could be ascertained by digging a small pit next to each rod. The rods were stored in air-tight plastic bags and assessed within 6 hours of removal from the soil. The depth to the anoxic layer was recorded as the difference between the soil surface position and the first unbroken area of uncorroded steel (grey colour). The soil surface position was adjusted to be 20 mm below the humus surface for the post-mature and open sites as this was approximately where the pots with roots were buried. Pole stage sites lacked a substantial humus layer and so no adjustments were necessary.