Acronyms and definitions

**15N** = Stable isotope of nitrogen, it represents the 0.3663% of the N in atmosphere.

**δ15N** = a measure of the ratio of 15N/14N of a sample compared to the ratio of the standard, i.e. the ratio of the atmospheric nitrogen, express as a permil (‰) value.

**DBH** = diameter at breast height.

**DIW** = deionised water.

**DM** = dried mass.

**EA-IRMS** = Elemental analysis – Isotope Ratio Mass Spectrometry).

**IQR** = interquartile.

**LSMSF** = Life Sciences Mass Spectrometry Facility.

**Ndep** = nitrogen deposition, input of reactive nitrogen from the atmosphere to the biosphere both as dry deposition and in precipitation, or wet deposition.

**SF** = stemflow, flow of intercepted water down the stem or trunk of a plant.

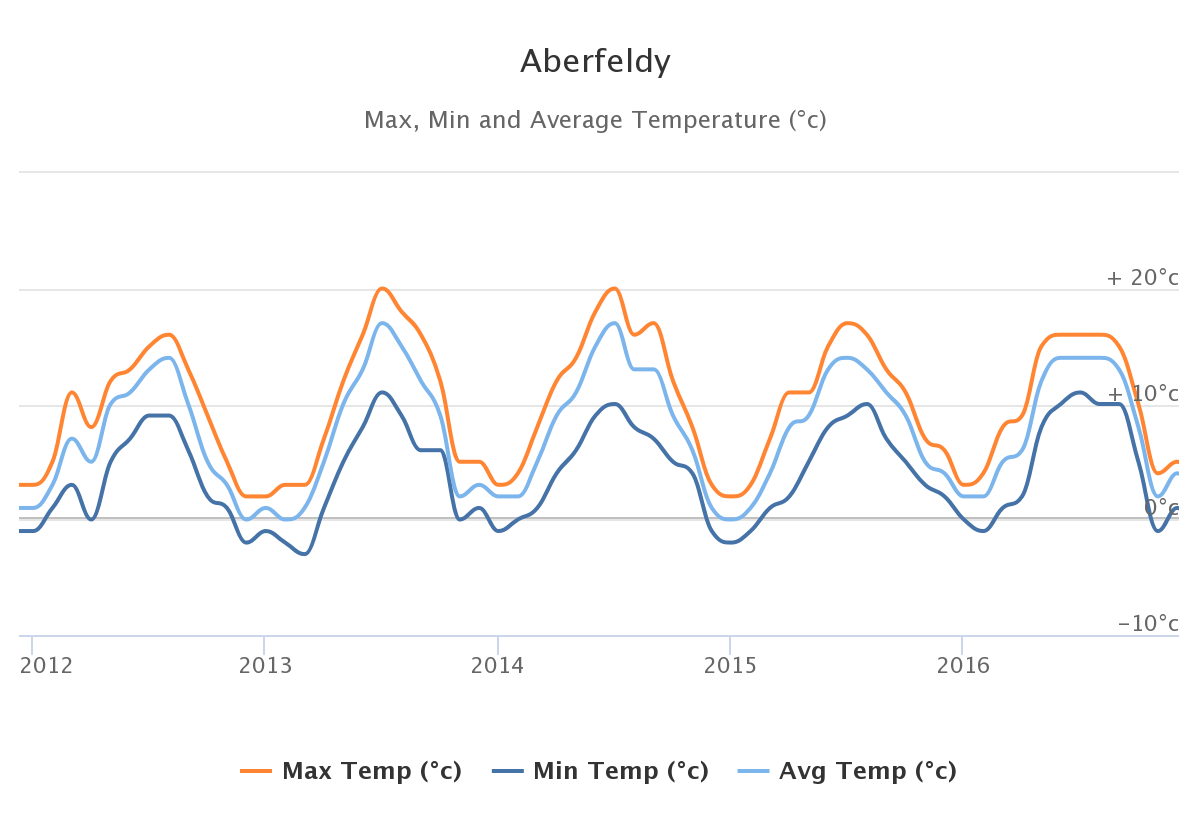
**TF** = throughfall, excess precipitation shed by wet leaves onto the ground.

# Chapter 2. Site description and methodology

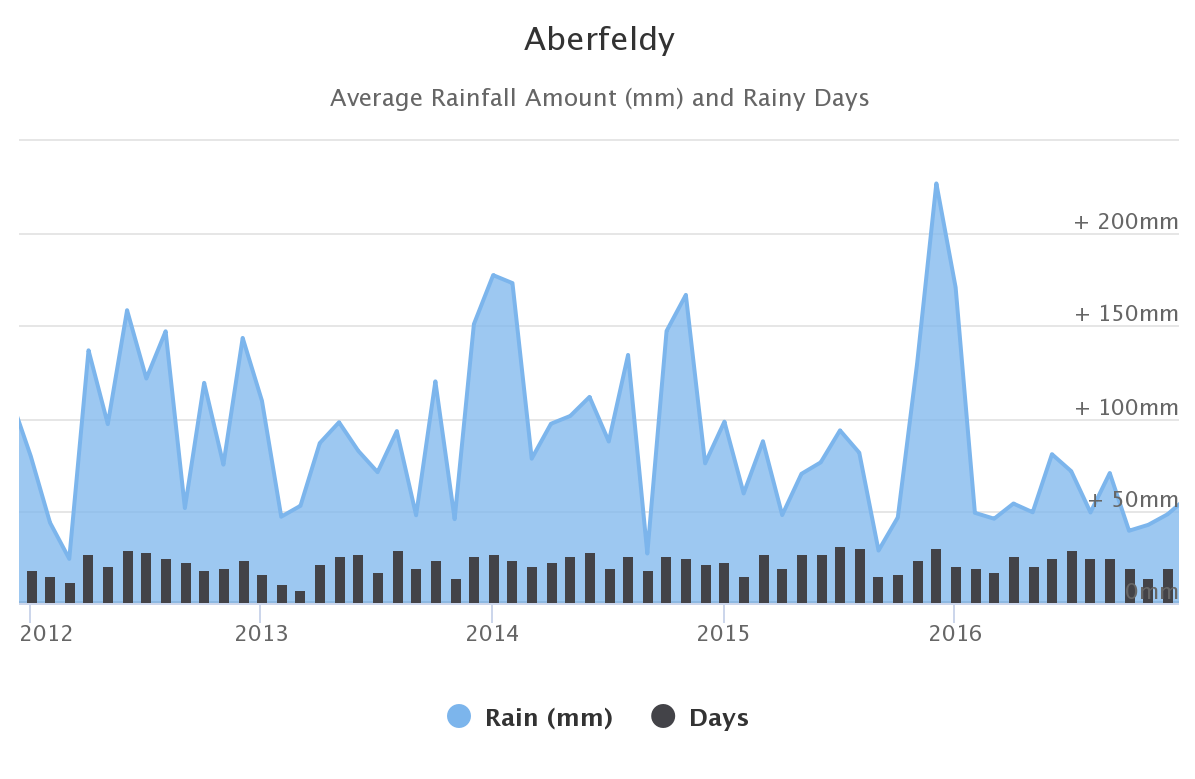
## Site description

The experiment site is located in Griffin Forest, Perthshire (Scotland, UK), on the north facing slope of the Tay Valley at about 4 km from the town of Aberfeldy.

Figures 1 shows the monthly average temperature for Aberfeldy. This area is characterised by relatively cool winters and warm summers, compared to Scotland. The average annual rainfall for the same period is about 1050 mm per year, with a maximum in 2014 of 1376.2 mm and a minimum in 2016 of 768.6 mm (fig. 2).



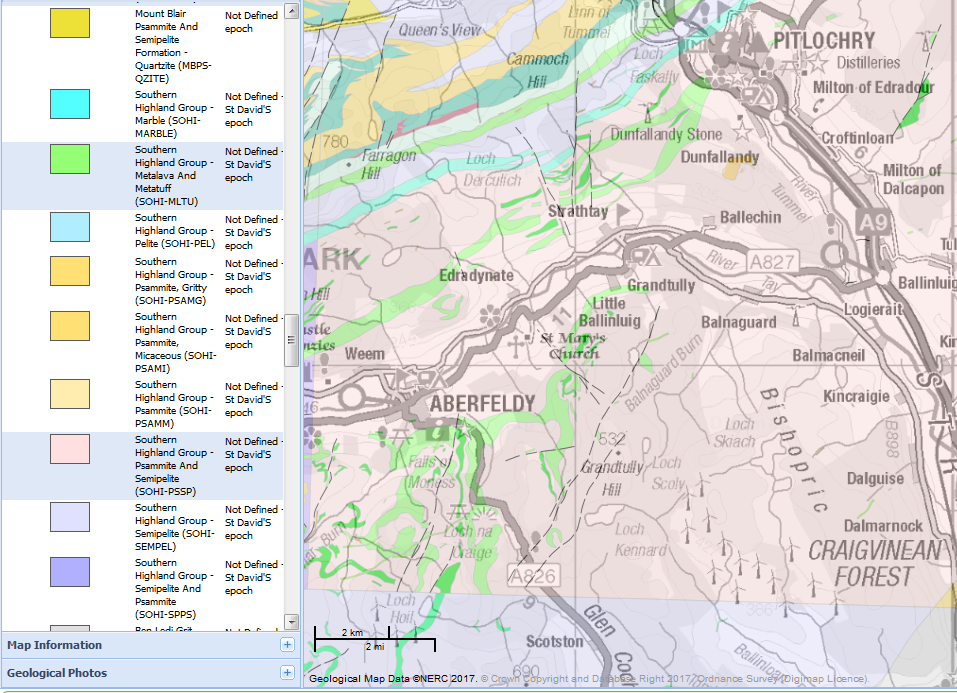
*Fig. 1: average monthly temperatures in Aberfeldy, years 2012-2016. Source: weatheronline.com*



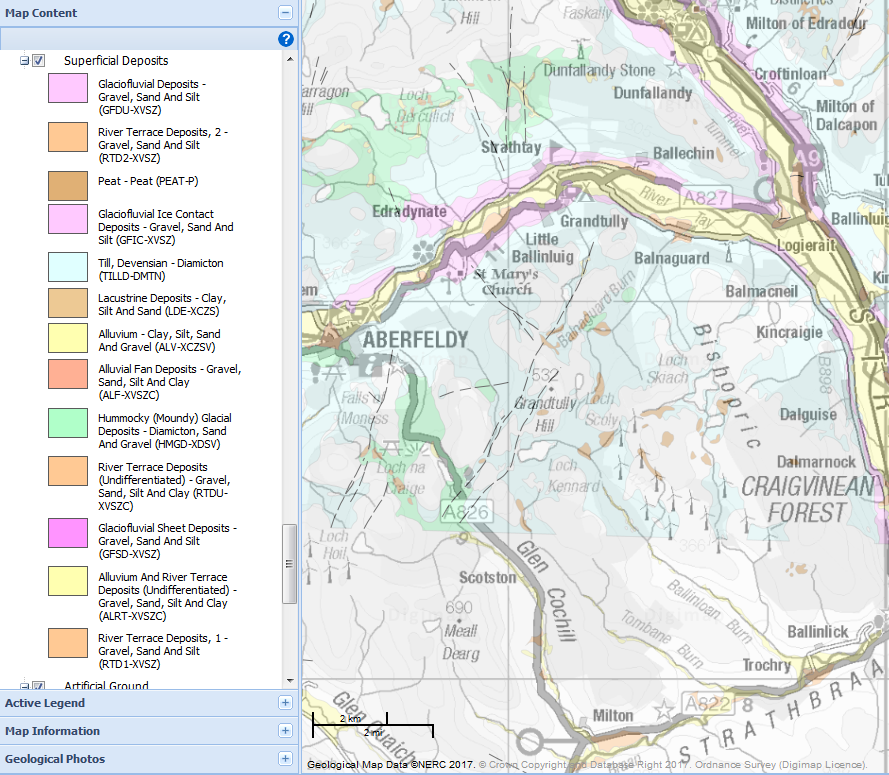
*Fig. 2: average monthly precipitation in Aberfeldy, years 2012-2016. Source: weatheronline.com*

Rocks in the area of Aberfeldy belong to the Dalradian supergroup, metamorphosed marine sediments of late-Precambrian and Lower Palaeozoic age (Craig 1925).

The bedrock under the forest is characterised by Psammite and Semipelite with minor inclusions of metalava and metatuff, covered by Devensian till deposits (see fig. 3 and 4). The soil have been classified as a stagno-humic gley of the Strichen soil association (Clement, et al. 2012).



*Fig:3 geological map of the bedrock in the Griffin Forest area (1:50000). Source: Edina digimap.*

**

*Fig:4 geological map of the superficial deposits in Griffin Forest area (1:50000). Source: Edina digimap.*

The main factors determining the maximum height at which trees can grow are altitude, climate (especially wind exposure) and soil quality. The potential maximum height of the tree line exceeds only slightly the 600 meters in the Cairngorms, north of the case study area, which is sensible lower than what can be found in Northern America and Scandinavia at similar latitudes. This can be due to the combination of the cool and wet oceanic climate and the wind exposure; soil types also indirectly reflect these two factors, as a direct combination of relieves and rainfall pattern. The formation of peat blankets in the Scottish uplands, started after the last glaciation, is another limiting factor to the growth of trees that has been changed with the use of drainages in plantations to make the growth of tree roots possible (Oosthoek 2013).

### Before afforestation: brief history of forest cover in Scotland

The present status of Scottish forest cover reflects a very recent afforestation process started on the second decade of the 20th century: at the start of the past century, in fact, less than 5% of the UK were covered by forests. When thinking of the Highlands, one might think that the typical world known scenery of naked mountains, whose rocks have been modelled by the force of Nature are the effect of the combined pressure of the climatic conditions and human pressure in a relatively recent past. In fact, after the last Ice Age different types of forests covered the Highlands, mainly pine and oak, except the extreme northern portion and the islands where the forest cover was mainly consisting of birch and hazel. The forests extent reached its maximum peak about 6,000 years ago, when an educated guess based on palynological investigations suggest that at least the 60% of Scotland was covered by some sort of vegetation. In that same era proofs of massive utilisation and deforestation dates 4000 years ago by the hand of early farming communities (Oosthoek 2013). In fact, whilst in north-west Scotland the pine forests were largely replaced by blanket-bog before any large impact by man, in the Eastern Highlands human activity was the main destructive agent, stretching over a period from about 1700 BC to about 1000 AD: the fire, the cattle, the sheep and the goats of the Highland peasants, aided by a period of climatic deterioration (the “Little Ice Age” started in the later fourteenth century and not really over before the late seventeenth century) reduced the estimated 50-60% of forest cover during the Mesolithic period to about the four percent during the Middle Ages (Smout 1997).

When the WWI burst about 182,000 ha of mostly broadleaved woodland were felled and the urge for a strategic policy to create and maintain a woodland stock brought to what can be considered the foundations of today’s forestry policy and practices: the Forestry Act of 1919. The Forestry Commission (FC) was created and a target of 0.75 million ha of new forests was set, so that the country had not to rely anymore on timber imports. Due to the second World War conflict, and the consequent need for more timber, in 1943 the FC set a new ambitious target: 1.2 million ha to be afforested and a further 0.8 million ha of “effective” forest to be created by restocking existing woodlands. Afforestation continued undisturbed despite of the loss of strategic interest. At the end of the 1970s the 1943 target was about to be passed. In December 1980 a ministerial statement announced a forestry policy that did not mention a total area of plantations anymore, but envisaged an afforestation rate of 20,000-25,000 ha per year, later increased to 30,000 ha in March 1986. Since 1919 upland afforestation has continued inexorably. Over the last 60 years nearly 18,000 ha have been planted each year.

The area of woodland in the UK at 31st March 2017 exceeds the 3 million hectares, 1.4 of which is located in Scotland. Conifers account for the 51% of the overall forest cover and almost three quarters of them are located in Scotland. Sitka spruce accounts for around a half of the conifer area in UK, with even higher figures in Scotland (58% according to the (Forestry Commission 2017)

### The plantation

“The vocabulary of an upland forester draws irresistible comparisons with agriculture. Afforestation of a bare hillside requires fencing, the elimination of wildlife such as hares and deer (regarded as vermin), deep ploughing and drainage, planting, fertilising and weeding. Today’s foresters talk of rotations, crops and harvests. Their crops have become steadily more dependent on a single, alien species, the Sitka spruce, with lodgepole pine used where the ground is poorest. Tree-breeding programmes are also ensuring that the genetic base of the plants used will steadily decrease. Blanket afforestation consists of even aged conifer monocultures that are harvested and replanted, like any crop of surplus grain, except that the trees take decades to grow.

The second basic ingredient of British forestry is that the rapid expansion of coniferous plantations has occurred on land where tree growth is least satisfactory – in the uplands. This was due to avoid competition with agriculture, as the production of food was seen to be of key importance, and to take place on cheap land and on a large scale, to reduce unit costs. As to 1986, 34% of hill, moor and rough grazing in Scotland had been afforested” (Tompkins 1989).

Since the Forest Census of 1995 the woodland area in Scotland is estimated to be grown of a further 7.5% (Forestry Commission 2011). In 1995, Sitka spruce represented about the 45% of the whole conifers in Tayside, over one third of the whole forest cover of the region.

The Griffin Forest plantation was established in 1980-1981 over an area classified as heather moorland. Before planting the trees heather (*Calluna vulgaris* (L.) Hull) was burnt and the ground was ploughed in depth, originating a surface characterised by 3 different features: ridge, furrow and undisturbed soil; ridges lay at 1.9 m from each other. Trees were planted at a distance of 1.9 m on the ridges, so that the total number of trees was of 2770 trees/ha (Clement, et al. 2012).

The about 4,000 ha were planted a dominance (80%) of Sitka spruce (*Picea sitchensis* (Bongard) Carriere 1855) and additional species such as Douglas fir ([*Pseudotsuga menziesii*](https://en.wikipedia.org/wiki/Douglas_fir) ([Mirb.](https://en.wikipedia.org/wiki/Charles-Fran%C3%A7ois_Brisseau_de_Mirbel)) [Franco](https://en.wikipedia.org/w/index.php?title=Jo%C3%A3o_Manuel_Antonio_do_Amaral_Franco&action=edit&redlink=1)), Japanese larch ([*Larix kaempferi*](https://en.wikipedia.org/wiki/Larix_kaempferi) (Lamb.) Carr.), Scots pine (*Pinus sylvestris* L.) and downy birch (*Betula pubescens* Ehrh.).

Thinning was carried out in 2003/4 on the east side of the forest and in 2005 on the west side by removing every 5th row and cutting every 3rd tree on the two rows aside of the thinned row; one third of trees were removed, bringing the present density to about 1750 trees per hectare.

This forest has been selected for a number of research projects mainly held by the University of Edinburgh and has been one of the case study areas of the EU-supported Euroflux project (Clement, et al. 2012), aimed to assess the role of European forests as carbon sinks over their life-time. One eddy covariance tower (plot T) from this experiment was left available and used during part of this study. Other master degree and honours dissertations provided useful information for the development of the methodology of this study.

## Experimental design

A first draft of the project aimed to compare two plots with similar features, one of them set as control plot (C) and a treatment plot (T) where a solution containing 15N-labelled NH4NO3 had to be sprayed over the canopy, similarly to Gaige et al (2017) experiment. Due to some constraints, the idea of the treatment was abandoned and the two plots have worked as a replica. Within the Griffin forest, two sub-basins have been set, at a similar altitude and orientation. T sub-basin is located at 56°36’22’’ N, 3**°**47’41’’ W, elevation ca.360 m; C plot is a sub-basin laying slightly downhill at 56°36’38’’ N, 3**°**47’40’’ W, elevation ca. 350 m.

In both plots 3 subplots were identified (coded as T10, T11, T12, C10, C11, C12), each of these covering similar features of forest cover, soil type, height and exposition. A storm in 2014 caused localised tree felling at the C site. As a result, even if the installed throughfall and stemflow collectors were not directly damaged, the C plot forest cover is locally less dense and could show slightly different soil and air temperature features than the T plot, especially in the subplots C10 and C11.

### Water and litter samples collection

The core of the experiment aims to compare the nitrogen input from the atmosphere to the nitrogen recovered below the canopy. Two rainfall gauges, one “harp-wire” fog collector (pictures?), 18 throughfall/litter and 22 stemflow collectors and 4 streamflow flux and sample collection points were set. The sample collection started on October 2011 and ended on April 2017.

Rainfall gauges. Two rainfall gauge was installed, one over the T plot (56°35'59.8"N 3°47'21.5"W, elevation: 440m) and a second one below the C plot (56°37'11.0"N 3°48'21.6"W, elevation: 286 m), both in open areas, sufficiently far from the plantation in order to minimise any turbulence due to the presence of high obstacles. In the upper rain gauge station a “harp-wire” collector was installed at short distance from the rainfall gauge. Rainfall and cloud water were collected through glass funnels (r = 7.5 cm) into plastic bottles; glass wool was placed in the mouth of each funnel as a filter for debris and other macro particles (pollen, dust, insects and bird dropping). In each of the rainfall bottles 2.5 ml of concentrated ortho-phosphoric acid was added as biocide agent (5 ml in the cloud water collection bottle due to the bigger capacity). No biocide agent was used in the throughfall and stemflow collectors, due to the high volumes of sample collected and the need to discharge in situ most of the sample, potentially causing a local increase of soil acidity.

Throughfall collection. 18 collection points have been set, 9 per each plot, 3 per each subplot, representing different positions within the canopy (thinning lines, full density and intermediate) and different wind directions. Throughfall is collected through two inclined gutters of a fixed length (4.02 m) and width (0.234 m) collecting throughfall and draining it to a barrel. The barrel is covered but lets the throughfall drain through a central colander (r = 12,25 cm) which works as a filter, retaining litter or other undesired objects or animals to pass into the barrel. The depth of the water collected is measured as close as possible from the centre of the barrel and turned into a volume through an algorithm formulated by testing in the labs each of the 3 barrel types deployed in field (small, round and square barrels). This volume is then turned into mean throughfall deposition by dividing each water volume by the total surface projection of the gutters and the colander, as follows:

Where

*n* = number of TF collectors

*Vi* = volume of the throughfall as calculated in the *i* barrel calculated through the calibration formula specific of each barrel type;

,

*w* = throughfall gutter width

*l* = throughfall gutter length

*α* = gutter angle

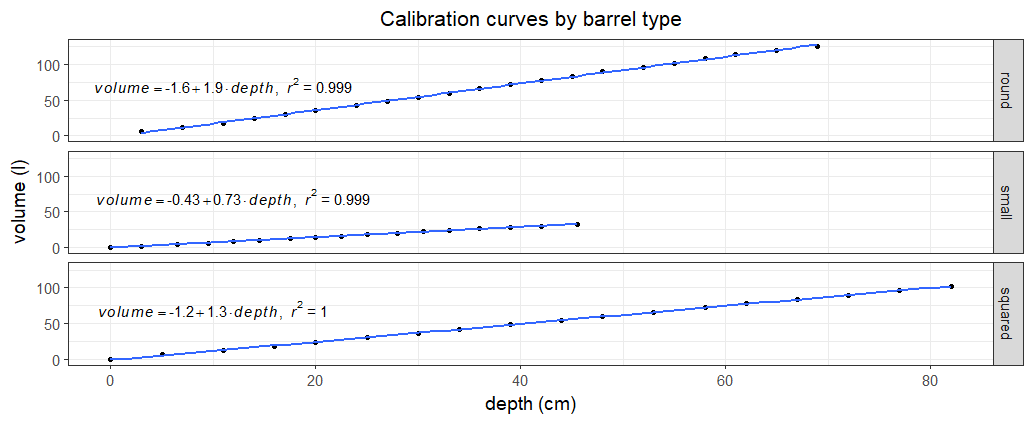
*r* = filtrating bowl radius

Litter collection. Litter was collected from the gutters and colander of the 18 TF samplers. Each individual sample was oven dried at 70 °C until no changes in weight were observed. Each sample was weighed and separately stored in sealed bags.

Stemflow samples. The rationale of the sample distribution follows the assumption that there is a linear relationship between (DBH)2 and stemflow volume as found by Heal, et al. (2004). The information on the plots DBH structure was gathered through a DBH survey conducted in 2011 in both plots - updated in February 2015. The surveyed trees have been divided in 8 DBH classes; 17 stemflow samplers were deployed in unthinned rows, representative of each of the 8 classes. 5 more samplers were deployed with the aim of assessing the effect of thinning: 3 were attached to the north edge of a thinned row, whilst other 2 were deployed in an unthinned row nearby.

The 22 trees selected represent this variability. 17 of them follow the distribution of the throughfall collectors. 5 extra collectors were deployed to better represent trees facing the thinning lines. The calculation of the volume collected is similar to what is done for the throughfall. The depth of the water in the barrel is transformed in a volume, then the mean volume of the samplers of each of the 8 classes is calculated, and the mean value of the 8 classes is scaled to the hectare by multiplying it for the number of the trees (1750/ha).

Throughfall and stemflow depth to volume conversion. 3 different types of barrels have been used. The small barrels, with a capacity of 30 litres, covered most of the stemflow samplers and the multiple throughfall collectors displayed in the 15N labelled Ndep simulation. The square and the round barrels have a capacity of over 100 litres of water and were used for the throughfall collectors. All collectors were calibrated in the labs and a linear regression for each barrel type (R2 ≥ 0.999) has been obtained (see fig. 5) and used to convert the water depth measured in the field into a volume.



*Figure 5: calibration curves for the barrels displayed in Griffin.*

For depths under the centimetre the volume was measured in field using the 250 ml Nalgene bottles.

Streamflow samples. 4 triangular-notch thin-plate weirs (British Standard 1981) were installed in Griffin, two in each plot. In the T plot one of them is up the plot and the second one collects the water outflow from the plot. This allows to calculate a net balance of the N leached by the plot. Although before the start of this study each weir was equipped with a pressure transducer attached to a datalogger in order to have a continuous flow measurement, all the stream flow data used in the present study are instant discharge as measured monthly during the sampling collection, when also stream water samples were taken for N concentration. This shows obviously a limitation to properly build up a hydrological balance but allows at least to have a rough information of the N leaching from the forest at different times of the year.

Water sample processing after collection

Samples were stored at 4 °C in the labs until the filtration, usually not more than 24 hours after the collection. A 60ml syringe was flushed out 3 times with 50 ml sample, then the same amount was taken from the Nalgene bottles (250 ml, 1 l for the streamwater) and filtered through EMD Millipore Millex™ Sterile Syringe Filters (0.45 μm pore size) into a biotite container, stored at 4 °C until analysed. Colorimetric analysis was performed at the University of Edinburgh laboratories to measure NH4+and NO3- concentrations. The remaining samples were stored at 4 °C until the lab results were available and rerun when outliers did not have a clear explanation (contamination due to known causes).

### 15N-labelled simulated Ndep

To confirm or reject the outcomes from the 5 years data collection a simulated Ndep was set on 3 Sitka spruce trees *in situ* by using 15N to label the applied solution and calculating the total recovery of the applied 15N in throughfall and stemflow. The trees have been selected around the existing eddy covariance tower in the T plot so the be easily reachable with an extension lance (the maximum reachable distance from the tower was 5.4 m) and were sprayed in two occasions (5th of August 2016 and 28th of February 2017) with a 15N-labelled enriched NH4NO3 solution (δ15N = +1000) by applying it over the tree crown from the top of the eddy covariance tower. Under each spruce 1 stemflow and 4 throughfall collectors were placed; these last would cover approximately 1/5 of the crown projection. The crown projection was estimated from the ground by measuring all distances from the tree to the surrounding trees or measuring the maximum extension of the branches when any of the surrounding trees were missing. Similarly to the 5 years collection, the depth of the water in each barrel was measured and samples of at least 5 litres (when available) of throughfall and stemflow were withdrawn. When the volume of the throughfall samples exceeded the maximum volume of the sampling tanks, 5 to 10 litres of sample were obtained by blending the 4 subsamples proportionally to the length of the water in each barrel. All samples were kept at room temperature for less than 24 hours since their collection, then refrigerated at 4 °C until the results from the colorimetric analysis were available. 20ml of samples were filtered and analysed through colorimetric analysis to measure the concentrations of NH4+ and NO3- at the Crew Laboratories at the University of Edinburgh, then enough sample was used to obtain 50-150 µg of NH4-N through the ammonia diffusion method (Sebilo, et al. 2004).

Ammonia diffusion method. With this technique, all NH4+and NO3- are converted into NH3 (gas) by means of pH adjustment and reduction (NO3- to NH4+) and entrapped into an acidified glass filter wrapped into a hydrophobic, gas permeable PTFE tape. Adjustment of the procedure in the paper were necessary so to treat up to 1 litre of sample, due to the usually low concentration of dissolved nitrogen in the throughfall and stemflow at natural abundance.

The incubation bottles have been filled with a 0.5M HCl solution, shaken for about 10 seconds and left to soak for 5 minutes, then rinsed with DIW 3 times and dried in a furnace at 400 °C for one hour. The preparation of the filter packages (Whatman™ Binder-Free Glass Microfiber Filters, Fischer Scientific, UK) was done on a clean aluminium foil wearing disposable gloves and by using scissors, tweezers and spatulas that were previously cleaned with ethanol. The microfiber filters were cut into 0.5 cm x 1.5 cm and left in the furnace at 400 °C for one hour. 30 μL of H2SO4 8N were pipetted on each filter, then the filter was wrapped and closed into a 19 mm PTFE hydrophobic tape by firmly pressing all sides with a spatula. A first set of filters was put into the incubation bottles with a variable amount of solution, calculated on the basis of previously obtained results from the colorimetric analysis of subsamples, so to contain between 50 and 150 μg of NH4+-N. NaOH (5M, 2 ml/150 ml of solution) was introduced and each bottle was immediately sealed with sealing parafilm, left for one week at room temperature and moved gently daily to remove any condensation drops from the walls or film. The incubation ensures the complete conversion of NH4+ into NH3 and its capture into the acidified filter. At this stage 300 mg of Devarda’s alloy were added to the samples in order to capture the NO3- by reducing it to NH4+, resealed and left for one more week, similarly to the first step.

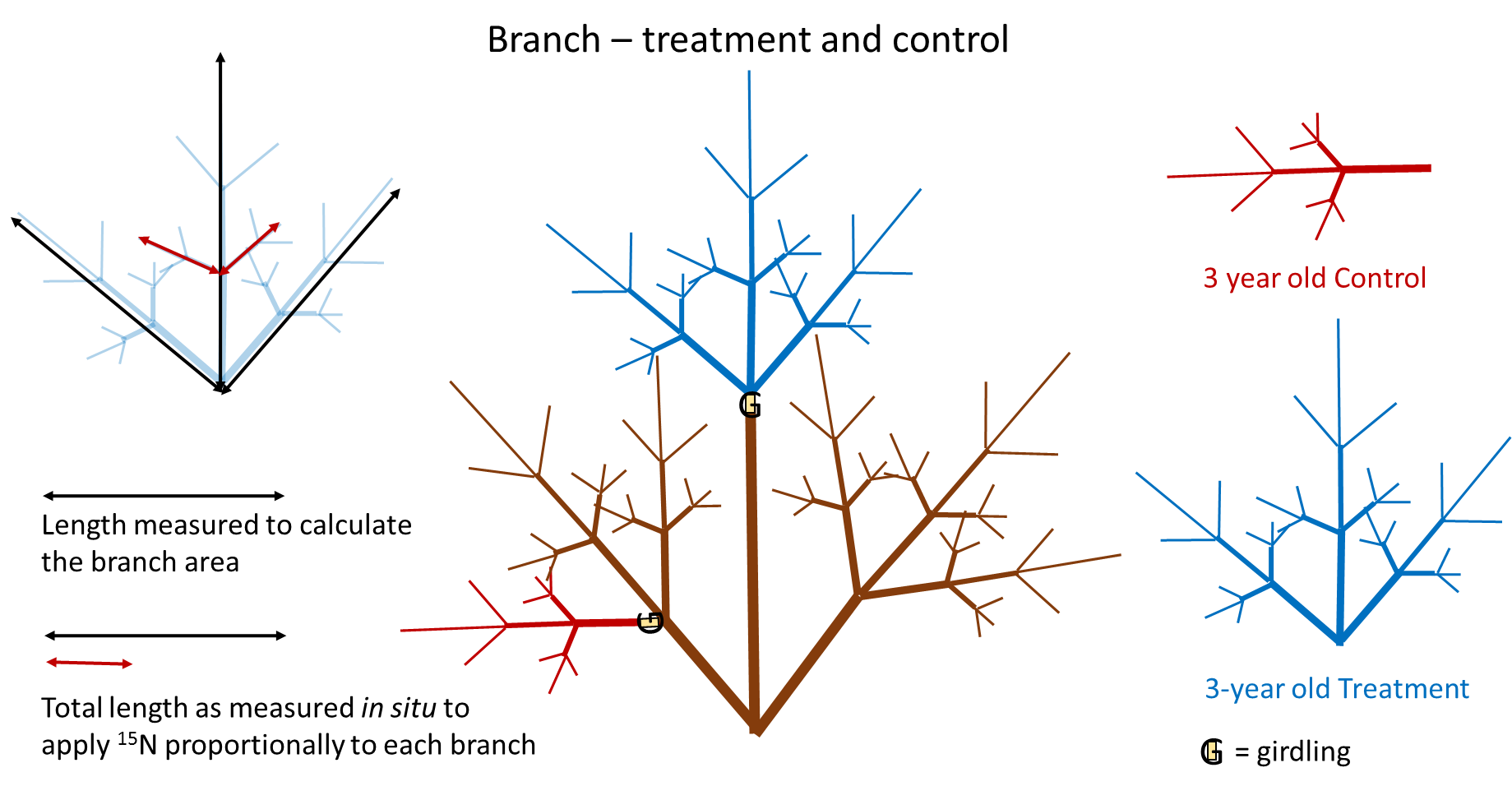
The obtained filters were frozen, then freeze dried at the Grant Laboratories of the University of Edinburgh and sent to the LSMSF (Lancaster, UK) for δ15N analysis through an EA-IRMS. COPIARE QUESTO ALLA FINE DEI SOILS ECCETTO IL FREEZE DRIED into a desiccator, put in 9x5 mm tin capsules (CE Instruments Ltd, UK)

### Direct nitrogen uptake from the crown: a 15N-labelled application on branches

Due to the different nature and relative abundance of N in the aqueous inflow and in the tree compartments, a separate experiment has been set to test the direct uptake of Ndep by the crown. A direct application of pure double 15N-NH4NO3 solution to some target branches has done similarly to what Nair, et al. (2015) had done on 3 years old Sitka spruce saplings, where they used 98% 15N double labelled NH4NO3 in a quantity (54g/ha/y) small enough not to increase significantly the total amount of natural Ndep.

10 branches were selected from 2 different trees in the T plot, easily reachable from the eddy covariance tower so to minimise losses of solution. For each branch 2 sub-branches were selected, one for the application and one for Control. The total amount of applied 15N was about 48 g/ha (9.73 mg applied on an estimated branch surface of 2.03 m2), so that it would not change the natural deposition conditions. They were distributed on 3 different layers on the crown, from about 20 to about 17 meters of height, being the last three levels of the eddy covariance tower. For the half of the branches, 2 hours after the first application a strip of phloem was removed from the base of the branch to avoid any translocation of N to other compartments of the tree. The total amount of 15N solution was applied in two rounds, on Saturday 6th of May, 2017 at 15:00, in dry, still and sunny conditions and on Sunday the 7th in the morning with similar weather conditions. All branches were removed in the late afternoon of Sunday and brought to the laboratories.

The branches were roughly measured in the field (see Fig. 6) so to apply an amount of 15N-NH4NO3 solution proportional to the size of the branches and twigs. Once removed, the total length of each branch and its twigs was measured to calculate how much 15N was applied per cm of twigs. All of the sampled twigs were measured, washed and rinsed 3 times with DIW the day after their collection in the field, then put in oven at 70 °C until dried. DM of the needles and twigs was weighed and a value of DM per cm of needles and twigs of each branch and age (new/old needles and twigs) was recorded. The dried samples were grinded on a Retch MM-200 ball mill, in metal capsules with a single ball, until a fine powder was produced, then weighed. A subsample of this powder was sent to the LSMSF (Lancaster, UK) for δ15N analysis through an EA-IRMS.



*Figure 6: branch representation and sampling rationale*

### 15N-labelled Sitka spruce litter mineralisation on distinct surface zones

4 plots of 15N-labelled Sitka Spruce litter were established in both the T and C catchments at Griffin (8 plots in total deployed) in summer 2013. Each plot covers the 3 typically different surface features under a ploughed upland plantation: undisturbed soil, ploughed furrows working as drainages and elevated ridges on which trees are planted and, representing respectively the 25%, 25%, and 50% of the total plantation surface (Conen, et al. 2005).

The litter was produced in a stem injection experiment (Nair, et al. 2014) where a labelled a double 15N-ammonium nitrate solution was introduced to 13 9-12m Sitka spruce edge-profile trees and left to distribute throughout the canopy. The resulting label was heterogeneously distributed throughout the foliage. In this litter deployment, the entire needle biomass from *outside* the stand (in total, 173 kg of litter) was deployed at Griffin. Each plot is centred on a single target tree with a 3 x 3 tree or about 4 m by 4 m plot edged by 8 additional trees. There was no significant understory in any of the labelled litter plots and virtually all of the litter derives from Sitka spruce canopy.

Due to the different mean 15N atom % of each injected tree litter, the plots received labelled litter at different average values of 15N atom % as follows:

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Litter Plot** | | **Treatment Location** | | **Control Location** | | **mass litter applied per plot (kg)** | **At Application average 15N atom %** | | **δ15N** | |
| A | | T11 | | C11 | | 22.1 | | | 1.67651 | +4655 |
| B | | T11 - T12 | | T11 - T12 | | 19.3 | | | 1.50631 | +4175 |
| C | | T12 | | T12 | | 20.6 | | | 1.66924 | +4634 |
| D | | T10 (downslope) | | C10 (downslope) | | 24.6 | | | 1.63818 | +4547 |

Soil samples were taken in two occasions, July 2015 and July 2017. The use of a soil auger was abandoned due to the risk of contamination through the layers due to the small diameter of the cores and the very high 15N concentration in the surface layer compared to the deeper layers. The cores were extracted by using a 15 cm wide blade spade, obtaining 15-20 cm squared cores, as deep as the position in the plantation le, usually about 20 cm or more in the ridges, 15 cm and less in the furrows, where strong soil erosion can occur due to their design as water drainages. 4 sets of 6 cores per each plot, 2 replicates per each surface type within each selected plot, were taken; 2 treated plot were selected among those located into labelled plots, 2 were controls, chosen nearby, 2 or 3 tree lines upstream. The soil cores were carefully removed, wrapped into transparent foil and brought back to the laboratories in a cool box and processed the following days. Some relevant differences between the two collections and processing are due to the water content of the soils in the two different dates and other constraints.

The first collection was done on the 15th of July 2015. T11T12 and T11 were selected, 6 soil cores were taken in each plot, two per each surface type (undisturbed, ridge and furrow). Similarly, 12 cores in total were taken as controls in similar position upstream of the two selected treated plots so to avoid any possible risk of cross contamination. The weather conditions were extremely wet and many soil cores were saturated with water (all of the cores from the furrows and undisturbed soil), so they were left at room temperature for two days before commencing the sieving. A central parallelepiped portion for each soil core was cut off to discard the fraction of sample where 15N contamination of the lower layers by the more enriched surface layers due to the action of the spade in field could happen; the squared shape also helped to calculate the soil density, as the portion disturbed by the spade and the extraction was eliminated at this stage. The core was then weighed, separated into layers and sieved through a 2mm mesh. Roots were separated at this stage, carefully washed and rinsed with DIW. Together with the litter layer, the >2mm fraction and the <2mm fraction they were dried at 80 °C until the mass showed to be stable and reweighed. All samples were milled in a stainless steel capsule on a Retsch MM400 ball mill (Retsch Ltd UK), until a fine powder was achieved, suitable for mass spectrometry. 5 gr (fresh soil) of each soil layer were shaken for 1 hour with 100 ml of 0.1M KCl, let them settle for 30 minutes and filtered through Whatman no.1 paper filter previously rinsed with about 5 ml of solution. The lab worked ended on the 31st of July, 15 days after the collection. *This could have caused partial fermentations and bacterial activity in the soil, due to the relatively high soil moisture even in the last cores that were processed.*

The second collection was carried out on the 5th of July 2007. The weather conditions the driest of the past 3 years, with most of the soil cores being ready for sieving. The decision to take only a partial sample for each soil core layers and roots let the samples to be processed in less than one week. In order to be able to cross the information from the first collection with the information coming from the gas flux chambers (see paragraph 2.2.5), installed at a side of plots T10 and C11, 6 soil cores – two per each surface type- were samples from each of the following plots: T10, T11, T11T12 and C11. 6 control soil cores were sampled from the T and C sub-basins, 12 in total, evenly distributed among the soil surface different features. Only samples from T10, T11, C11 and all the control cores from the T sub-basin were processed.

### Gas flux chambers and N2O fluxes

18 closed chambers were installed in Griffin under the supervision of the Forest Research, 9 per each plot, 3 replicates per each surface type (ridge, furrow, undisturbed soil). Gas sample from the air-tight samples were taken from October 2013 to November 2016 monthly, except in the winter months where snow cover or ice in the air tubes and in the water frames made the sampling unfeasible. The technique follows Ryden and Rolston (1983). Before the start of each sampling air temperature, soil temperature and soil moisture were annotated. 4 sets of two replicates were taken from each chamber every 20 minutes from minute 0 to minute 60 after the sealing. The vials were put into a cool box and sent to the Forest Research (Alice Holt Lodge, Surrey, UK) for the analysis. From (dates) soil cores were also taken in proximity to the chambers, then KCl extraction was carried out. The obtained samples were analysed at the University of Edinburgh through colorimetric analysis and NH4+ and NO3- concentrations were measured.

### Data Analysis

Data analysis was carried out using R and several packages under the RStudio™ development environment. The fieldwork and lab results were copied and saved into separated monthly Windows Excel datasheets, imported and saved into a RSQL database after some basic calculations were done: turning water depth in the barrels into water volumes (TF and SF) and TF depth (mm), rainfall samples (as weight) into precipitation (mm), subtracting the mean values of blanks to the N concentration results. All the data have been analysed for outliers (through the Tuckey’s method ranged above and below the 1,5\*IQR, where necessary predicted through regression or interpolation (1 case out of 60 months collection) and saved into a second RSQL database into a daily value. All the results as tables, plots and statistics were created in R importing data from the RSQL database, unless stated differently.

For the 15N labelled experiments the fieldwork and lab results were saved into Microsoft .csv files, then imported and analysed in RStudio™. *(further explanation on soil data to be added)*

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