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.

**FC** = Forestry Commission

**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.

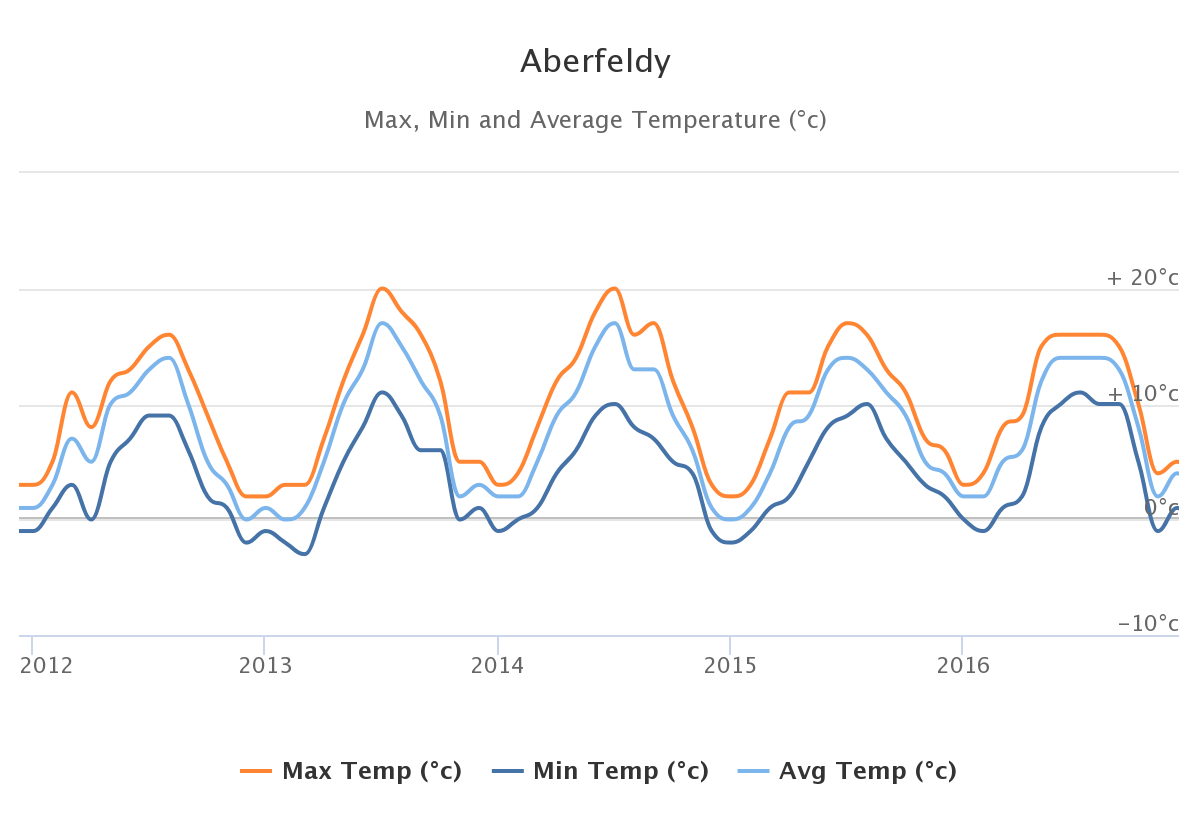
**TF** = throughfall, excess precipitation shed by wet leaves onto the ground.

# Site description and methodology

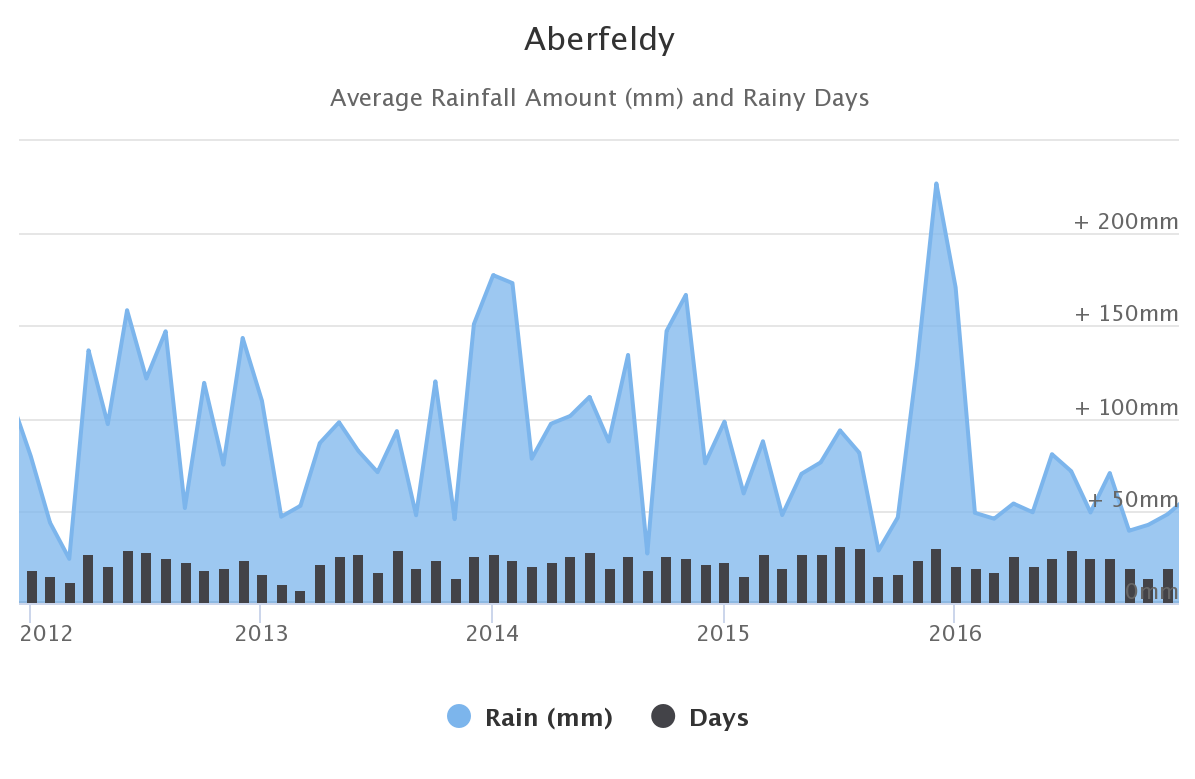
## Site description

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 of768.6 mm (fig. 2).



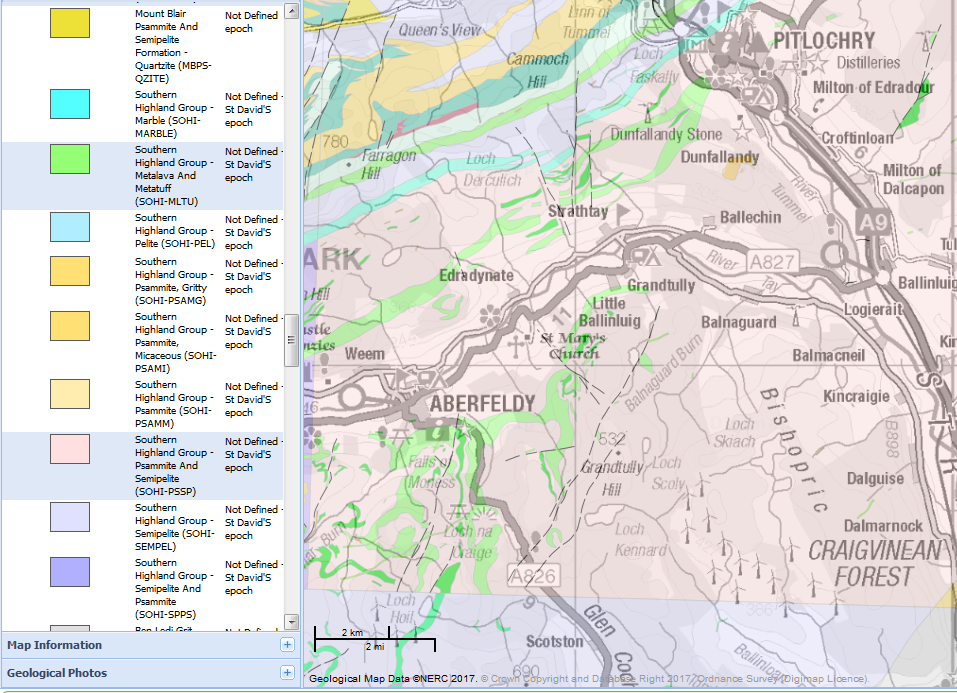
*Fig. 1: average monthly temperatures in Aberfeldy, years 2012-2016. Source: weatheronline.com*



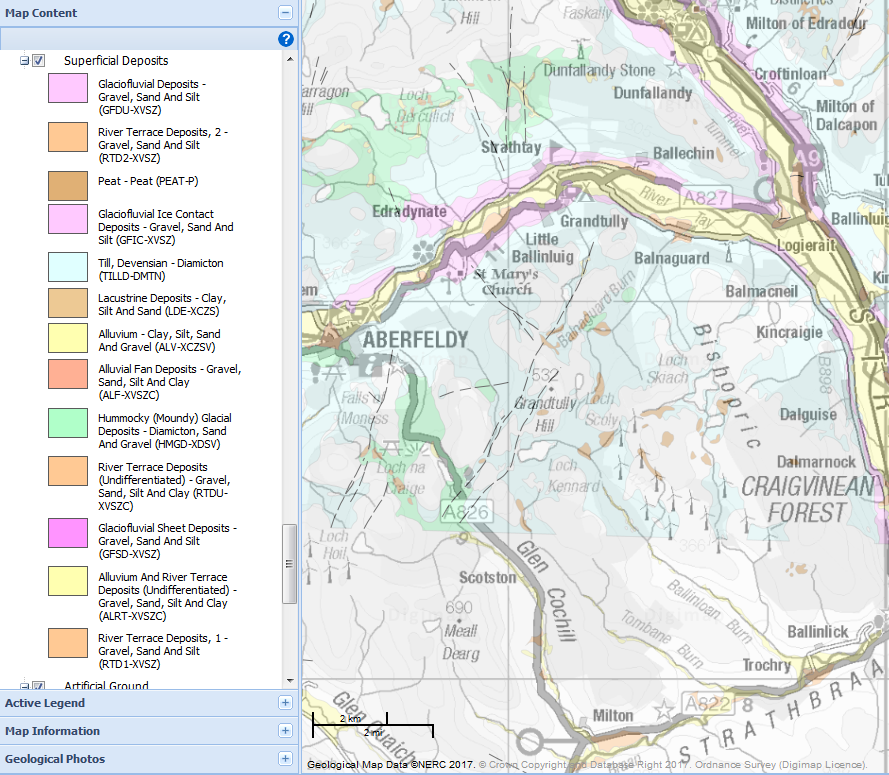
*Fig. 2: average monthly precipitation in Aberfeldy, years 2012-2016. Source: weatheronline.com*

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, Jarvis, & Moncrieff, 2012).



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

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*Fig:3 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).

As written above, at the beginning of the 20th century forests counted for about 5% of the total land in UK. 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 Wolrd 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 in Scotland. Sitka spruce accounts for around a half of the conifer area in UK, about 58% in Scotland (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" \o "Douglas fir)* ([Mirb.](https://en.wikipedia.org/wiki/Charles-Fran%C3%A7ois_Brisseau_de_Mirbel" \o "Charles-François 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" \o "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.

## Experimental design

Separate description for each experiment here or to be restructured on an “experiment” base?

### The plots

The originary 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 become a replica. Within the Griffin forest, two plots have been set, at a similar altitude and orientation.

T plot: 56°36’22’’ N, 3**°**47’41’’ W

C plot: 56°36’38’’ N, 3**°**47’40’’ W

In each of the plot 3 subplots were identified, each of these covering similar features of forest cover. A storm in 2014? caused a notable damage at the C site. As a result, even if the installed collectors did not suffer damages, the C plot forest cover is locally less dense and could show different soil and air temperature than the T plot, especially in the subplots C10 and C11.

### Water and litter samples collection

The core of the experiment aimed(s) 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 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) in two open areas, sufficiently far from the plantation so to minimise any turbulence due to the presence of high obstacles. In the upper rain gauge station the “harp-wire” collector was installed at short distance from the rainfall gauge. In each of the bottles 2.5 ml (5 ml in the bigger bottle for the cloud water collection) of concentrated ortho-phosphoric acid was added as biocide agent. 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.

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 stored in sealed bags.

Stemflow samples. Kate doc. 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, Stidson, Dickey, Cape, and Heal (2004) the information gathered through a DBH survey conducted in 2011 in both plots - updated in winter 2016\*. The surveyed trees have been divided in 8 classes; 17stemflow 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).

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 each weir was equipped with a \*\*\* sensor and a \*\*\* in order to have a continuous measurement (check Rob maybe? Or Thesis), eventually the available data, instant discharge and N concentration, come from the monthly collection. This shows obviously a limitation to properly build up a hydrological balance but allows at least to have an information of the N leaching from the plots in 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. 30 ml of each sample were withdrawn from the Nalgene bottles (250 ml, 1 l for the streamwater) with a 60ml syringe after rinsing it 3 times with the same amount of sample, filtered through EMD Millipore Millex™ Sterile Syringe Filters (0.45 μm pore size), stored at 4 °C until analysed. Colorimetric analysis was performed at the University of Edinburgh laboratories to measure NH4+and NO3- concentrations.

### Experiment “15N-labelled simulated Ndep”

This experiment aims to confirm or reject the outcomes from the 5 years data collection by simulating Ndep by using 15N as a label and calculating the total recovery of the applied 15N in throughfall and stemflow. 3 Sitka spruce trees have been selected around the existing eddy covariance tower in the T plot so the be easily reachable with an extension lance (maximum length = 5.4 m) and were sprayed in two occasions (\*\*dates) with a 15N-labelled enriched NH4NO3 solution (δ15N = +1000) by applying it over the tree crown. Under each spruce one stemflow and four throughfall collectors were placed so to cover approximately 1/5 of the crown projection. Similarly to the existing experiment, the depth of the water in each barrel was measured and a sample of at least 5 liters (when available) of throughfall and stemflow were withdrawn. When the volume of the sample exceeded the volume of the sampling 5 to 10 liters 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 kept at 4 °C until the results from the colorimetric analysis were available. In the labs 20ml of samples were filtered and analysed through colorimetric analysis to measure the concentrations of NH4+ and NO3-, then enough sample was used to obtain 50-150 µg of NH4-N through the ammonia diffusion method (Sebilo, Mayer, Grably, Billiou, & Mariotti, 2004). Adjustment of the procedure in the paper were necessary so to treat up to 1 litre of sample, due to the low concentration of dissolved nitrogen in the throughfall and stemflow at natural abundance. 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.

The obtained filters were frozen dried at the Grant Laboratories of the University of Edinburgh and sent to the \*\*\* for δ15N analysis.

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

In a simulation of Ndep on a nursery experiment on Sitka spruce saplings, Nair, Perks, Weatherall, Baggs, and Mencuccini (2015) used 98% 15N double labelled NH4NO3 in a quantity (54g/ha/y) small enough not to increase significantly the total amount of natural deposition.

Due to the different nature and relative abundance of N in the aqueous inflow and in the tree compartments, the quantity of 15N previously applied to the canopy would be difficult to be discriminated by the possible differences due to fractionation processes; 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 previously done on 3 years old Sitka spruce saplings. 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 15N was \*48 g/ha, so that it would not change the \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

### 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: ploughed furrows working as drainages, elevated ridges on which trees are planted and undisturbed soil, representing respectively the 25%, 50% and 25% of the total plantation surface (Conen et al., 2005).

The litter was produced in a stem injection experiment (Nair, Weatherall, Perks, & Mencuccini, 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 by 4 m plot edged by 8 additional trees. There was no significant understory in any of the labelled litter plots and all of the litter derives from Sitka spruce canopy.

Richard: “These treatments received 15N labelled litter (at 1.53 ± 0.4 (s.e.) atom % 15N

(3227 15N h), 1.87 ± 0.1 atom % 15N (4184 15N h), , and 2.09 ± 0.2 atom% 15N (4807 15N h). A second treatment received unlabelled litter, at natural abundance(0.366 % 15N h) for all plots). As this labelled litter was derived from multiple trees (three per plot), injected with 15N (Nair et al. (In Press), in press.), we minimised the within-treatment variance in the 15N content by mixingthe litter for each plot well before application. Total dry masses of 15N-labelledlitter deployed were 23.0 kg, 22.2 kg, and 21.7 kg, and unlabelled litter 29.81 kg 29.52 kg and 27.07 kg. Trasforma la tabella sotto in dati cosi’.

Due to the different mean 15N atom % of each injected tree litter, the plots showed 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 %** |
| A | T11 | C11 | 22.1 | 1.67651 |
| B | between T11 and T12 | between T11 and T12 | 19.3 | 1.50631 |
| C | T12 | T12 | 20.6 | 1.66924 |
| D | T10 (downslope) | C10 (downslope) | 24.6 | 1.63818 |

Soil samples were took in two occasions, July 2015 and July 2017 using a 15 cm wide blade spade, obtaining 15-20 cm squared cores, as deep as the position in the plantation let (20 cm and more in the ridges, 15 cm and less in the furrows, where strong soil erosion can occur). 4 sets of 6 cores per each plot, 2 replicates per each surface type, were taken; 2 were 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 coolbox and processed the following day.

1st campaing. A central parallelepiped portion for each soil core was cut off to avoid the 15N contamination of the lower layers by the more enriched surface layers due to the action of the spade in field. The core was then weighed, separated into layers, to establish the density per each layer, and sieved through a 2mm mesh. Roots were separated at this stage, carefully washed and rinsed with DIW. Together with the >2mm fraction and the <2mm fraction they were dried at 80 °C until the mass showed to be stable and reweighed. All litter, roots and soil 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 shacken 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.

2nd campaign.

### 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 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.

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