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**Effects of Experimental Drought on Fine Root Biomass in Two
Different Altitudinal Forests of Bhutan**

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Submitted by

Yonten Dorji

Supervisor

Univ.Prof. Ph.D. Dr. Douglas L. Godbold

Institute of Forest Ecology, UNI BOKU

Co-supervisor

Iftekhar Uddin Ahmed, MSc. Ph.D

Institute of Forest Ecology, UNI BOKU

Declaration

I hereby declare that this master's thesis is my own work and effort. It has not been submitted in any other University for any other award. Due acknowledgements have been made to all materials cited in the text.

Place: Gregor Mendel Straße 33, 1180 Wein

Yonten Dorji

Date: 6th August 2015

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Abstract

The severity of the impacts of changes in South Asian monsoon climate is a highlighted topic of current scientific debate. Fine root system is recognised as a major reservoir of carbon (C), which contribute to global terrestrial C cycle substantially. However, there are few studies on belowground tree biomass compared to above ground in the Himalayan region. Here in this research, a manipulated drought experiment was carried out to investigate the effect of the moisture stress on the fine root biomass, in two forest types along the altitudinal gradient in the Himalayan Bhutan. We hypothesised that: 1) soil moisture stress can significantly decrease the fine root biomass of these forests, 2) soil rewetting after removal of the roof, leads to significant recovery of fine root biomass. Root biomass was estimated during three sampling intervals viz. before roofing in May, after roofing in August and recovery period in November, following a sequential coring approach. We estimated 466 to 627 g m⁻² fine root biomass in the forest of higher and lower altitude, respectively. Our results showed no significant decrease in fine root biomass due to through fall exclusion for four months. And there was no significant change in fine root biomass during the recovery period. Therefore, we conclude that, there is no effect of the moisture stress on the fine root biomass of the two forest types of Bhutan. However, we could see a decreasing trend of fine root biomass in both of the control and roof plots, indicating a seasonal impact during the summer season. A long term drought experiment is necessary to comprehensively observe and quantify the effect of the seasons on the fine root biomass.

Key Words: Monsoon climate, Forest carbon, Fine root biomass, Drought experiment.

Kurzfassung

Der Ausmaß der Auswirkungen von Änderungen des Monsunklimas in Südasien ist ein wichtiges Forschungsthema unserer Zeit. Die Feinwurzelmasse in Wäldern ist ein wichtiger Kohlenstoffspeicher, was erheblich zum globalen terrestrischen Kohlenstoffkreislauf beiträgt. In der Himalaja Region gibt es im Vergleich zu Studien über oberirdische Biomasse dennoch wenige Studien, die unterirdische Biomasse untersuchen. Diese Studie verwendete ein manipuliertes Experiment, um die Effekte von Trockenstress auf die Feinwurzelbiomasse in zwei Waldgesellschaften entlang eines Höhengradienten in Bhutan zu untersuchen. Die Hypothesen waren, dass 1) Trockenstress signifikant die Wurzelmasse in diesen Wäldern verringert, und 2) Wiederbefeuchten des Bodens nach Entfernung des Daches führt zu einer signifikanten Erholung der Feinwurzelbiomasse. Die Wurzelbiomasse wurde dreimal (vor dem Aufbau des Daches im Mai, nach dem Aufbau des Daches im August, und während der Erholungsphase im November) mit Hilfe von sequentieller Bohrungen beprobt. Die geschätzte Feinwurzelbiomasse beträgt am oberen Standort $466 \text{ g}\cdot\text{m}^{-2}$ und am unteren Standort $627 \text{ g}\cdot\text{m}^{-2}$. Die Ergebnisse zeigen keine Abnahme der Feinwurzelbiomasse aufgrund der Überdachung während einer Periode von vier Monaten. Ebenso wurde keine Änderung der Feinwurzelbiomasse während der Erholungsphase beobachtet. Daraus wird geschlossen, dass Trockenstress keinen Effekt auf die Feinwurzelbiomasse in den beiden Waldgesellschaften aufweist. Eine abnehmende Entwicklung der Feinwurzelbiomasse über die Vegetationsperiode unabhängig von der Behandlung deutet auf einen saisonalen Effekt während des Sommers. Ein Langzeit-trockenstressexperiment ist nötig, um saisonale Effekte von Trockenstress auf die Feinwurzelbiomasse im Detail zu untersuchen.

Schlagworte: Monsunklima, Kohlenstoff, Feinwurzelbiomasse, Trockenstressexperiment

1 Introduction

1.1 State of art and problem statement

The Himalayan region is one of the most vulnerable to adverse impacts of climate change. Climate change is expected to adversely affect natural ecosystems, composition, functions, biodiversity and human livelihoods in the long term (Shrestha et al., 2012). Temperature increases in the Himalayas are predicted to be three times greater than the global average, however there is very limited understanding how these affects the ecosystems (Shrestha et al., 2012). Isotope chronologies in Nepal suggest an increasing aridity over past two centuries in the Himalayas (Sano et al., 2011). Cook et al. (2010) developed a Monsoon Asia Drought Atlas, with spatial information on mega droughts, which underlines the potential importance of future drought phenomenon on ecosystem and livelihood in this region. Increased precipitation has been found to stimulate plant growth and ecosystem carbon fluxes, whereas decreased precipitation has the reverse effects (Bréda et al., 2006b). The impacts of the reduced precipitation are more pronounced in arid ecosystems compared to other mesic ecosystems (Wu et al., 2011). Decreased precipitation suppresses Aboveground Net Primary Productivity (ANPP), Net Ecosystem Exchange (NEE), soil respiration, ecosystem photosynthesis and net carbon uptake. Response of NEE and ANPP are more sensitive to increased precipitation than to decreased precipitation (Wu et al., 2011).

Bhutan is situated approximately between 26°45' to 28°10' North latitude and 88°45' to 92°10' East longitude in the eastern Himalayas. The forest is the single largest resources of the country, and covers 70% of the country's area (Norbu, 2000), Bhutan has committed to maintain at least 60% of the forest cover for in perpetuity (Constitution, Kingdom of Bhutan). The altitude ranges from 100m in the south to above 7500m in the north within a distance of around 150km (Baillie et al., 2004). Consequently, there are different types of forest ranging from strong moisture gradient subtropical forest in the south, temperate and broad leaved forest in the central area, to dry sub-alpine forest in the northern area (Wangda and Ohsawa, 2006).

Drought is one of the major causes for climate change induced tree mortality (Adams et al., 2009). Drought events are likely to synergistically increase the risk of biotic disturbances, like bark beetle attacks, and increase the frequency and intensity of forest fires (Westerling et al., 2006). Developing and planning strategy for increasing the resilience against climate change risks is a major determinant for the future consequence of climate change on

livelihoods (Lobell et al., 2008). Especially for countries like Bhutan with very steep, erodible terrain and low economic resilience, this holds also true for forests. Potential changes in the disturbance regime needs to be characterized and knowledge of how to increase resilience of different forest ecosystem is critically important. This would require a better understanding of stress tolerance, including below ground functional biodiversity associated with the main tree species.

Fine roots (≤ 2 mm diameter) are responsible for water and nutrient uptake and therefore have a central role in carbon, nutrient and water cycling, at the plant and ecosystem level. Fine roots biomass represents about 30% of the above-ground biomass, and between 40-85% of the net primary production according to soil type (Hoffmann and Usoltsev, 2001). Fine roots sustain soil biological activity, and also influence soil organic matter and nutrient dynamics (Hendricks et al., 2006). Fine root systems contribute substantially to global terrestrial carbon (C) cycle, and are a major reservoir of C (Vogt et al., 1998). However there are few studies in annual fine root production compared to above ground production. This is, as annual fine root production is difficult to measure and challenging. Thus less is known about physiological and environmental control of root parameters (Olesinski et al., 2011). The ability to predict forest responses to climate change will be improved with greater understanding of the fine root dynamics, therefore it is important to assess whether and to what extent fine roots in Bhutanese forests change with climate.

1.2 Through-fall Exclusion Experiment (TFE) or Drought simulation

According to Wu et al. (2011), the future precipitation regimes are expected to be very highly variable and unpredictable, thus long term experiments and studies addressing structural changes in ecosystem are needed. Therefore, field experiments with precipitation manipulations are required to elucidate the impacts on ecosystem. Soil drought simulation or the through fall exclusion (TFE) experiments are a widely used method for assessing drought stress in forest stands. These experiments are carried out to study the impacts of the droughts stress for different forest types, over extensive areas. Many studies have been carried out in the Amazonian rain forests using this soil water drought simulation (Phillips et al., 2009, Nepstad et al., 2007a).

In this current study, a through fall exclusion experiments (TFE) was used to characterize the ecosystem responses triggered by altered precipitation patterns for key forest types along an altitudinal gradient in the Bhutan Himalayas. The work is focused on root biomass of the

dominant species of the forest types. This study is part of the long-term ecosystem study being carried out by the Renewable Natural Resources Research Development Centre (RNR-RDC), Department of Forests and Park Services, Royal Government of Bhutan and The University of Natural Resources and Applied life Sciences, Austria. The experiment mimics the total monsoon failure.

1.3 Objectives and Hypothesis

The main objective of the research is to examine the effects of water stress on fine root biomass in a manipulated drought experiment along an altitudinal gradient of Himalayan Bhutan. The specific objectives for experiments are as follows:

- i) To compare the growth of fine root biomass in control and roofing treatment plots across three time periods of; a) before roofing, b) after roofing and c) recovery period.
- ii) To study the effects of drought on the growth of fine roots of hemlock dominated coniferous and oak dominated mixed broadleaved forests.
- iii) To analyse the depth wise distribution of the root biomass of the dominant species in the two forest types.

The main hypothesis of the research is that, the decreased soil moisture will significantly decrease the fine root biomass in both the forest types. Secondly, soil rewetting after removal of the roof leads to a significant recovery of fine root biomass.

2 Literature Reviews

2.1 Global Climate change trends and its impacts

Temperature and precipitation are two key drivers of ecosystem processes (Diodato et al., 2012). It was found that there will be a considerable climate change by the end of the 21st century; the global mean temperature is anticipated to increase by 2-7°C and precipitation regimes change globally (Eriksson et al., 2009). Ecosystem carbon balance will be altered as a result of increasing climate change (Wu et al., 2011). A change in precipitation patterns is characterized by increased variability and greater extremity and is the least predictable driver. The effects of changes in precipitation on the ecosystem is not well understood (Fan et al., 2009). Further, climate change is gradually increasing severe drought events worldwide and causing severe tree mortality and diminishing the efficiency of forests as carbon sinks globally (Carnicer et al., 2011, Martínez-Vilalta et al., 2012). Carbon, energy and water balance will be affected, and thereby alter plant communities, population dynamics and disturbance regimes (Beier et al., 2012). Over the recent decades, effect of climate change on the ecosystem functioning has received much focus (Gautam et al., 2013). However most studies have focused mainly on the effects of rising atmospheric CO₂ levels and increasing temperatures (Wu et al., 2011). The effects of changing precipitation on ecosystem processes have received less attention and have been studied only recently. Although changes in precipitation are correlated with ecosystem C fluxes and plant productivity, responses of different ecosystems is still unclear (Wu et al., 2011).

2.2 Forests of Bhutan

Geology: Bhutan has a geology, which is fragile and calls for conservative management. There are huge differences between the northern and central regions and the southern fringes. Folded and metamorphosed rocks of Precambrian and early Palaeozoic age, largely quartzite and gneiss, cover most of the country. The soils of Bhutan have good permeability and moderate moisture retention. Forest soils are of good tilt although generally shallow. The terrains are rugged and hence maintenance of vegetative cover and careful use of soils is necessary to check erosion and landslides (Sargent et al., 1985).

Bhutan is one of the most rugged and mountainous countries in the world. The elevation varies from 150 meters above mean sea level in the south to more than 7500 m in the north. Bhutan is divided into three altitudinal regions; i) The Himalayan region is a distinct biogeographic zone lying above 4500 meters altitude, ii) The temperate region is between 500 or

1,000 m and 4,500 m altitude, iii) The subtropical region consists of southern foothills below 1,000 m and river valleys below 500 m altitude.

The Land Use Planning Project (Land Use Planning et al., 1997) of Bhutan provides the information about forest and other forms of land use. The Land Use Planning Project (LUPP) has identified nine broad forest types, which are briefly described below:

Fir forest: The Fir forests are found on the highest ridges, ranging between 2700 m and 3800 m. These forests require high precipitation, part of which is obtained as condensation. Vegetation is characterised by a thick layer of moss with rhododendron, sub-alpine bamboo, and *Bryocarpum hamalaicum* as the undergrowth. Few hemlocks (*Tsuga dumosa*) and birches may also be present. And towards the tree line (at 3600 to 3800 m) the fir forests become stunted and grade into juniper and rhododendron scrub.

Mixed conifer forest: The mixed conifer forests lies between 2000 m and 2700 m altitude and covers an area of about 486,710 ha. It occupies the largest portion of the sub-alpine regions of the country. The dominant species found are spruce (*Picea spinulosa*), hemlock (*Tsuga dumosa*) and larch (*Larix griffithii*). The undergrowth consists of rhododendrons, bamboo and other shrubs.

Blue pine forest: The blue pine forests lies between 1800 m and 3000 m in the temperate regions. The blue pine (*Pinus wallichiana*) is dominant species and demonstrates fast colonization. It is sometimes found mixed with oak (*Quercus griffithii*) and rhododendron (*Rhododendron arboreum*).

Chir pine (*Pinus roxburghii*) forest

The Chir pine forests are found at an altitude between 900 and 1800 m in the deep, dry valleys under sub-tropical conditions and cover about 100,870 ha. These areas experiences a long dry season and the annual precipitation (1000-1300 mm) fall primarily during the summer monsoon.

Broadleaved mixed with conifer: This forest consists of oak (*Quercus griffithii*, *Quercus lanata*) mixed with blue pine (*Pinus wallichiana*), or upper hill forest mixed with *Picea spinulosa* or *Tsuga dumosa*. It occupies a total area in this forest type is about 135,770 ha.

Evergreen oak forests: These are more common in the drier areas and lies between 2000-2900m. Maple (*Acer campbellii*) and *Castonopsis spp.* predominate at lower altitudes, while oak predominates higher up. With increasing altitude these forests grades towards blue pine.

Cool broad-leaved forests: The lowland hardwood forests occupy the sub-tropical hills between 1000-2000 m altitudes and are very rich in species of both sub-tropical and temperate genera. The tropical lowland forests are multi-storeyed, are very rich in species diversity, and found on the low hills below 700 m. These forests are broadly classified as semi-evergreen but vary from almost totally deciduous on exposed dry slopes to almost totally evergreen in the forest valleys.

Forest scrub: The forest scrub includes alpine and temperate scrub occurring naturally between the limits of the tree line and barren rocks and covering an area of about 325,730 ha. The dwarf juniper (*Juniper squamata*), *Rhododendron setosum* and *Rhododendron lepidotum* are common species in the forest scrub.

2.3 Climate change in monsoon climates, with special reference to Bhutan: what is the state of Art?

2.3.1 Climate Change in the Himalayas

The Himalayan regions are known as the ‘third pole’ (Schild, 2008) and the ‘water tower of Asia’ (Xu et al., 2009). It has the largest snow and ice cover in the world apart from Polar Regions, the Himalayas directly or indirectly affects the livelihood of 300 million people (Schild, 2008). The concerns of the climate changes in the Himalayas are multifaceted, it encompasses drought, landslides, floods, biodiversity, endangered species, human health, agriculture livelihood, and food security (Xu et al., 2009).

In a regional study done by Diodato et al. (2012), using the Climate Research Unit, reconstructed a temperature dataset, they showed that in the last few decades, the Himalayan and Tibetan plateau regions have warmed at a rate higher than that in the last century. Further, the data analysed from 90 stations showed that, between the years 1961 to 2003, there has been an increase in warming over the whole Tibetan plateau, at the rate of 0.36⁰C per decade (Wang et al., 2008). Other research studies with proxy temperature data reconstructed from tree ring width, have also confirmed warming in the Tibetan Plateau (Cook et al., 2013). For precipitation, most of the literature reports a lack of spatially consistent long-term trends in Himalayan precipitation, however a statistically significant downward trend in monsoon and average annual rainfall has been observed in the northwest Indian Himalaya during 1961 to 2006 (Bhutiya et al., 2010). A recent study done by Scherler et al. (2011), analysed 286 mountain glaciers across the Himalayan regions and they found that, more than 65% of glaciers in the monsoon-influenced regions are retreating with

several heavily debris-covered glaciers. Scherler et al. (2011) attribute these phenomenons to increasing temperature and decreasing precipitation.

In the long run, climate change is expected to adversely affect natural ecosystems, composition and functions, biodiversity and ultimately human livelihoods (Xu et al., 2009, Shrestha et al., 2012). Despite these severe effects, the exact changes in temperature and precipitation are not well understood. The severity of the impacts of climate change and monsoon climatic changes is a frequently highlighted topic of current scientific debate.

Most studies about climate change revolve around the retreat of glaciers while studies on temperature and precipitation trends are very limited. These studies report that the temperature rise in the Himalayas is three times greater than the global average, however, regional changes are little explored, and there is very limited understanding of the variations in temperature and precipitation within regions. According to (Shrestha et al., 2012), the effects of these important parameters on the ecosystems is also not known.

2.3.2 Monsoon failure and changes in precipitation and temperature regimes: Observations and Evidences

The Asian monsoon is linked with changes in the large-scale atmospheric circulation over substantial parts of Asia. These massive changes are driven by the differential seasonal diabatic heating of the Asian continent and the tropical Indo-Pacific Ocean (Fan et al., 2009). The effects of this on water resources over these densely populated Asian region is large, therefore it is of significant importance to understand the possible future effects of climate change on the Asian summer monsoon (Meehl and Arblaster, 2003). The El Niño–Southern Oscillation (ENSO) has an effect on the monsoon by changing the zonal pattern of moisture transport in the Indo-Pacific linked with the Walker circulation (Nigam, 1994). In fact, a major portion of the historical monsoon failures have been identified with strong El Niño events (Grove, 1998). Further, a constant weakening of the Walker Circulation in the Pacific is associated to failure of Indian summer monsoon. All mega drought events are reported to have occurred during the severe El Niño events during which there is a maximum warming in the central Pacific (Nigam, 1994). Monsoon failures and extreme droughts have been occurring since the last millennium and has continually affected the farming community of Asia (Cook et al., 2010).

Drought reconstruction is also one of methods of understanding the past monsoon failure events and probable future regimes. A recent reconstruction of droughts in the ‘south-eastern

Tibetan plateau over the past 568 years by Fan et al. (2009), show close similarities with the drought incidence in regions of Indochina. They reveal an occurrence of decadal dry intervals and severe wet eras from the 15th till the late 20th Century. They also found that tree rings have a positive correlation with precipitation and negative correlation with temperature (Fan et al., 2009).

The spatio-temporal variability of Asian monsoon systems is not well understood, despite its vital importance. In their effort to understand this, Cook et al. (2010), developed the Monsoon Asia Drought Atlas (MADA) using tree ring chronologies from more than 300 sites. Using this, they showed that in the late twentieth century, India and Southeast Asia were relatively drier and experienced weaker monsoons.

Climate history reconstruction using paleo-climate proxies like tree rings and ice cores are useful tool for understanding the past climatic variations, Bao et al. (2003), showed that stable oxygen isotope variations of the Purogangri glaciers can be used to characterize altered summer temperatures for the central Tibetan Plateau. This enabled them to envisage future climatic change and probable scenarios. An increasing trend of stable oxygen isotopes indicating constant warming in the Tibetan Plateau in the last century was observed. These trends ascertain the reliability of the historic temperatures resulting from ice cores. However, ambiguities still remain, with regard to temperatures changes and their variations and there is a need for more analysis and study to ascertain these claims (Bao et al., 2003).

The different circulation systems have some influence on the temperature variance over the regions. Bao et al. (2003), in their analysis of the past reconstructed temperature changes in the Tibetan Plateau reports that different periods of warming and cooling occurred over the Tibetan Plateau. These periods were greatly influenced by the winter monsoon, and the south west and south east summer monsoons over the region. A reconstruction of the past summer temperatures of East Asia using tree ring chronologies by Cook et al. (2013) also provide some insights on the variability of temperatures. They also reported that the average temperature of the late 1990s were much greater than the temperatures of the past warm periods. However, this reconstructions lack statistical certainty to back up the findings. More studies in this field are required (Cook et al., 2013).

2.3.3 Causes of Monsoon Failure and Assessment

Rising temperatures trigger shifts in monsoon patterns, and the global atmospheric circulation systems influence the monsoon systems (Xu et al., 2009). The main cause of mean Indian

summer monsoon variations is the intervals in global mean temperature and the weakening of the Pacific Walker circulation. Global warming is anticipated to increase the monsoon rainfall due to increased moisture content in the atmosphere; however, “weakening of the Walker Circulation” during summers in the tropical Pacific will lead to more frequent failures of the Indian summer monsoon (Schewe and Levermann, 2012).

Precipitation data in the region are inconsistent, in some areas it has increased while in others a decreasing trend is reported (Xu et al., 2009). Menon et al. (2013) reported that the annual mean Indian monsoon precipitation increases with rising temperatures in their recent analysis of model projections, and also showed that there is an increasing trend in the inter-annual variability under future warming. This further demonstrates an association between increasing global temperatures and the Indian monsoon rainfall. Monsoon failure will be a predominant phenomenon as a result of constant warming. According to Schewe and Levermann (2012), a “statistically predictive model” developed for future monsoon failure in India have shown that monsoon failure will be a common phenomenon under increased warming trends, and will be an unusual event under pre-industrial conditions. Increasing incidences of delayed monsoon onset are anticipated in India. The state of the Walker circulation is reported as an important predictor for the model. Schewe and Levermann (2012) also reported that the end of 21st and 22nd centuries will experience more frequent monsoon failures.

2.4 The global role of drought as a disturbance agent in forests, and its relevance in terms of climate change effects.

2.4.1 What is Drought?

Drought occurs in all climatic zones, and are generally related to the reduction in the amount of precipitation over a region for a time period (Mishra and Singh, 2010). However, droughts, being a natural disaster, are characterised by multiple climatological and hydrological parameters. Hence, a common accurate definition of drought is not possible, due to the difference in hydro-meteorological variables and socioeconomic factors, as well as the stochastic nature of water demands globally (Mishra and Singh, 2010). The type of drought and the nature of shortage, such as stream flow, precipitation, soil moisture and groundwater levels have to be considered while defining droughts. There are four categories of droughts i) meteorological drought, ii) an agriculture drought, iii) stream-flow drought and v) ground water drought (Vrochidou et al., 2013).

Due to increasing demand for water and threatening global warming, global drought scenarios have seen much focus recently (Mishra and Singh, 2010, Vrochidou et al., 2013). Droughts are acknowledged and considered as a natural hazard and have become a major concern among scientists of different disciplines. Drought stress occurs when the soil humidity and the relative atmospheric humidity are low, and the temperature is high. Droughts occur, when there is discrepancy of the water flux between potential evapotranspiration and water flow in the soil-root system (Lipiec et al., 2013). Drought is frequently referred to as a ‘creeping phenomenon’ owing to its cumulative impacts over a period (Mishra and Singh, 2010).

2.4.2 Role of Drought in forest ecosystem

Climate change is gradually increasing severe drought events worldwide and causing massive tree mortality and diminishing the efficiency of forests as carbon sinks globally (Carnicer et al., 2011, Martínez-Vilalta et al., 2012). Tree mortality in turn, will change the forest structure, composition, forest carbon dynamics and flammability. Several drought episodes would also accelerate vegetation shifts from woody trees to grassland due to high mortality and fire outbreaks (Nepstad et al., 2007a). Drought has significant effects for below ground carbon (C) and nutrient cycling. It is also expected to impact the soil processes, through alteration in carbon allocation to roots and leaves as well as carbon yield in the rhizosphere (Sanaullah et al., 2011).

Increased droughts and changes in fire regimes, cause major changes in the vegetation structure. These changes are more prominent in the ecotones (Allen et al., 2010), and will cause considerable changes in ecosystem services, including wildlife habitat, water resources, provisioning services, soil formation, nutrient cycling, carbon cycling and recreational services (DeSantis et al., 2011). A recent study of tree mortality associated with drought and heat stress by Allen et al. (2010), showed that 88 drought events occurred worldwide in the past 30 years. This finding indicates that, droughts pose a great threat to the forests. This kind of large-scale tree mortality, in turn is expected to potentially affect carbon, energy and water balance, and also alter plant communities and population dynamics (Martínez-Vilalta et al., 2012). Droughts are expected to stimulate large-scale mortality of trees in temperate forests, by reducing the soil water availability below the threshold level. This restricts growth and overall transpiration and thus lowers the survival chances of the tree (Bréda et al., 2006b). Under reduced water availability, climate change is expected to aggravate the plant

susceptibility to attacks by groups of insects, Thus causing excessive defoliation and increased tree mortality (Jamieson et al., 2012).

Drought is also reported to alter the distribution of carbon among leaf, root, wood growth, carbohydrate storage and fruiting in the plants (Martínez-Vilalta et al., 2012). The carbon cycles have a great impact on the biodiversity (da Silva, 2012, Nepstad et al., 2007b). Phillips et al. (2009) in their study to assess response to droughts, reported that Amazon forests are more vulnerable to droughts, with a huge potential of carbon losses. They also report that this will also aggravate climate change in the Amazon forests. Da Silva et al. (2012), also show that droughts have significant negative effect on the tree growth parameters and decrease biomass. A considerable decrease ($21.1 \text{ t ha}^{-1}\text{yr}^{-1}$) of biomass was observed in the Amazon rainforests for the 2005-2009 period.

Droughts and heat waves together are also expected to impact the soils and its functions by changing the biomass, composition and activities of the edaphic microbial communities (Berard et al., 2011). In their recent study of resilience of soil microbial communities to severe droughts and high temperature, Berard et al. (2011), observed that soil microbial communities restructured with a change in their physiological characters. Berard et al. (2011) further report that the microbial catabolic functions were diminished, which will affect ecosystem processes, such as carbon mineralization and sequestration.

2.4.3 Importance of studying Drought

As a result of spatio-temporal changes in climatic patterns and increasing demand for water, various regions over the globe have experienced frequent droughts in the last three decades (Mishra and Singh, 2010). IPCC scenarios predict for the South-east Asian region to have a probability of a 12-month drought once in a century (Sheffield and Wood, 2008). Severe drought and increased heat stress is expected to fundamentally change the composition, structure and biogeography of forests worldwide (Allen et al., 2010). Allen et al. (2010) further suggest risks to ecosystem services, including the loss of carbon and atmospheric feedbacks. These ecosystem services are crucial for sustaining the ecosystem themselves, as well as the socioeconomic wellbeing of society.

Thus, understanding the effects of drought will enable better management of the forest ecosystem, and diminish the impacts on human society in the future. Allen et al. (2010), in their review, report that all forests globally are susceptible to the anthropogenic climate change, including regions in which water stress is not an issue. Increased mortality rates

observed and reported in extensive studies globally indicate that the forested ecosystems may be already shifting as a result of climate change. Thus, understanding, large-scale tree mortality induced by droughts and effects of climate change, is crucial for influencing and supporting policy decisions and forest management practices in many regions, for ensuring the ecological and societal well-being (Mishra and Singh, 2010).

2.4.4 Physiology of Drought

Drought stress results whenever soil water reduces below a threshold level. Decreased water availability changes both soil-root and leaf-atmosphere interfaces and also impedes the wholeness of the liquid phase continuum from soil to leaves (Eshel and Beeckman, 2013). Water fluxes along the soil-tree-atmosphere continuum are directly regulated by potential evapotranspiration, which is mainly irradiance, and vapour pressure deficit (Bréda et al., 2006b). The decrease in soil water content causes the hydraulic resistance within the soil and the soil-root interface to increase (Eshel and Beeckman, 2013). Due to decline in soil water content in most tree species, stomata closure occurs and this limits the water fluxes in the plants (Chaves et al., 2003). Increase in the drought intensity cause irreversible disruptions of steady state water transfer in xylem, which is detrimental to tree survival (Bréda et al., 2006a).

2.4.5 Plants Response to Drought

It is also very important to understand the various responses of plants to drought conditions. Various characteristics in plants enable them to adapt to varying water stress conditions (Eshel and Beeckman, 2013). Some of the crucial traits of the plants, which aid in adapting to stress are phenology (Chaves et al., 2003), size and depths of root system (Moreno-Chacón and Lusk, 2004), xylem properties (Bréda et al., 2006b) and the storage reserves (Allen et al., 2010). In the nature, plants can experience two type of stress, slowly developing water stress (within days, weeks or months) and short-term water stress (hours to days) (Chaves et al., 2003). Plants can withstand dehydration, in case of slowly developing water stress, by completing their life cycle within a short time before the occurrence of physiological water deficit (Pallardy, 2010). In case of rapid dehydration, plants respond by reducing water loss or by protecting metabolic activity (Pallardy and Rhoads, 1993). Plants can also withstand extreme water stress by preventing tissue dehydration or tolerating low tissue water potential (Phillips et al., 2009). These traits are mostly exhibited by annuals and perennials species.

The most adaptive traits in these plants are minimizing water loss through transpiration and maximizing water absorption in roots (Chaves et al., 2003).

2.5 The role of tree roots

Plant roots have been the plant-soil interface since the beginning of land plant existence. They play a vital role in water and mineral acquisition, and are essential for plant growth and development. They have the function of tapping and developing new resource structures, and act as a pipeline system for the aboveground biomass to provide it with water and dissolved nutrients (Eshel and Beeckman, 2013). Feedback cycles ensure an on-going monitoring of environmental parameters. Under the conditions of drought, for instance, roots can adapt in such a way as to continue growing, while at the same time producing and sending early warning signals to shoots, which inhibit plant growth above ground (Sanaullah et al., 2011). The chemical signals are initiated by specific root genes, these signals even control whether or not leaves grow (Eshel and Beeckman, 2013). As the outermost tissue that covers the growing root apex, the root cap plays a central role in the perception of such signals (Barlow, 2002).

Another important function of tree roots is their ability to anchor the individual tree in the soil matrix. The weight of emergent trees can be well over ten tons, and these forces must be absorbed by the belowground roots (Eshel and Beeckman, 2013). External influences like wind can stress the tree architecture to its limit; therefore, evolution has developed a variety of root systems that are highly efficient in the absorption of forces, for instance, overturning by wind or by failure of the root system under the stress of gravity is probably the most common cause of death in mature rain-forest on both level ground and steep slopes (Eshel and Beeckman, 2013), it is very likely a priori that adaptation increasing the mechanical stability of the tree will have survival value (Pallardy, 2010). Beside the primary functions that concern the individual tree, there are numerous secondary purposes served by tree roots. Such benefits are the protection against soil erosion and the triggering of dynamic soil processes, like the function of acquiring nutrient elements from deeper to shallower horizons, and thus counteracting leaching (Näsholm et al., 2009). Heavy soil profiles with relatively weak aeration can limit root growth, and root turnover creates soil channels which can improve soil aeration and water interflow (Eshel and Beeckman, 2013).

2.6 Root study

Global climate change scenarios and its relationship with plant growth, and the role of forest to increase the sequestration of carbon in ecosystems to reduce atmospheric carbon dioxide levels have generated a much interest in study and research of forest biomass (Ravindranath and Ostwald, 2007). Forests have been suggested to play an important role in controlling global carbon pools and fluxes (Vogt et al., 1995). Since root production contributes about half of the carbon being cycled annually in many forests (Vogt et al., 1995) and 33% of the global annual net primary production (Jackson et al., 1997), getting an accurate estimates of belowground biomass are important. Neilson (1995) suggest that, it is high time that root biomass should not be treated as a black box, or predicted using relationships developed in other ecosystems for scaling up in global modelling efforts

Root systems are fundamental components of terrestrial ecosystems, as the belowground net primary productivity ranges from 40 to 85% of the total Net Primary Productivity (Scurlock and Olson, 2002). However, methodological and analytical problems affect all root studies. Roots are the ‘hidden half’ of most terrestrial ecosystems (Eshel and Beeckman, 2013), therefore the researcher’s ability to design sampling protocols has been limited by the inability to visibly monitor the dynamics of an entire root system of a plant. The difficulty faced in root studies, is in having to design sampling protocols without having prior information on the root distribution patterns, phenology or seasonality of root growth or how root biomass or its morphological characteristics respond to the abiotic environments or to a changing environment (Vogt et al., 1998). One of the present challenges in root study is to improve techniques and methods to assess root system structure (Blouin et al., 2007). Most of the controversy for estimating fine root dynamics revolves around the estimates of production and turnover, and how different abiotic resources could change these parameters (Gower et al., 1992). One of the reasons for controversies in estimating fine root production and turnover in forests is that trees have highly variable allocations of photosynthate to fine roots, varying from 4–69% of total plant carbon annually fixed, which can significantly affect the ecosystem-level processes (Vogt et al., 1995).

Root studies are usually very laborious and time consuming, consequently, the process of planning such a study is especially important. Many methods must be adapted to the specific study aim and hence it is very difficult to give any indication about the labour and time use. Such indications are very seldom found in the literature (Bohm, 1979), hence it could be necessary to conduct test studies or analyse literature where similar methods have been used

to make any estimation for the time and labour use. “Generally it can be said that the more accurate the method, the more laborious it is” (Bohm, 1979).

There is a wide range of methods for root investigation found in various literatures. It is quite clear that every method has its own advantages and disadvantages. Most methods are based on destructive sampling, mainly soil coring (Rytter, 1999). The attempt to classify the methods has spawned problems in the past and in the present because several methods have certain similar features. The sequential root coring method is the most commonly used method to collect root biomass data in the past and presently too, but within the last decade the use of minirhizotrons has become popular among many researchers (Vogt et al., 1998). Sometimes it is advisable or even necessary to use two methods simultaneously (Bohm, 1979). A paper by Vogt et al., (1998) discusses and compares the results of the most commonly used direct and indirect methods of determining root biomass and production, the methods mentioned are i) sequential root coring, ii) ingrowth cores, iii) minirhizotrons, iii) carbon fluxes approach, iv) nitrogen budget approach and correlations with abiotic resources. They found, there were no apparent consistent relationships when comparing several sites, where at least one of the indirect and direct methods was used on the same site. The paper concludes that one root method cannot be stated to be the best, and ultimately the method of choice will be determined from researcher’s personal preference, experiences, equipment, and finances (Vogt et al., 1998).

2.7 Fine Roots biomass and drought

Fine roots play an important role in uptake of nutrients and water by being the primary pathways. Fine roots are also an important sink for carbon acquired in terrestrial net primary productivity (Hendrick and Pregitzer, 1996). Primary production allocated below ground is often greater than that allocated above ground, and annual carbon and nutrient inputs to the soil from fine roots frequently equal or exceed those from leaves (Jackson et al., 1997). Despite the importance of fine roots for nutrient cycling, resource capture, and global biogeochemistry, fine roots are poorly represented in global models. The lack of representation of fine root data is in sharp contrast to the prevalence of canopy leaf area data as an important input (Neilson, 1995).

Jackson et al. (1997) developed a biogeochemical model to provide the first global estimates for fine root biomass, length, surface area, and nutrient contents and their distribution with depth in the soil. They found that the total fine-root carbon pool is 5% of the size of the

atmospheric carbon pool, and the biomass of living fine roots represents 33% of annual net primary productivity. The potential changes in fine root production and turnover due to climate change, could change the nutrient availability in forest soils, and in turn influence the overall production and feedback to climate change (Ruess et al., 2006) . Therefore, accurate estimates of fine root biomass, production, turnover, and nutrient pools are important for improving and refining C budget models (Yuan and Chen, 2010).

Fine root production is susceptible to environmental stresses throughout the growing season, although the degree of vulnerability may vary seasonally with growth rate (Joslin et al., 2000). Soil temperature and the coordination with the shoot growth is the cause for variability in fine root production seasonally (Pregitzer et al., 2000). The paper by Olesinski et al. (2011), reports that in a cool, moist climates of balsam fir forests, fine root production was found to be lowest in spring because of low temperature and partitioning of photosynthates to shoot growth, and highest in summer season when temperatures are warm and there is little internal competition for carbohydrates, and lower in autumn as the temperatures decline. Further field experiments that compared droughted and undroughted plots, reported that the fine root production is reduced when drought treatment was applied in the summer, but no reduction was reported when the drought was applied early in the growing season (Joslin et al., 2000, Gaul et al., 2008). In a replicated through fall exclusion experiment in an Indonesian rainforest carried out by Moser et al. (2014), they reported a reduction of 35% of fine root biomass after 25 months of soil desiccation, and an increase in mortality rate of fine roots: Fine root necromass increased by 250%.

The ability of ecosystems to survive and adapt to changes in climate will depend in part on how individual trees allocate resources to their various plant components. It has been well documented that tree species that have adapted to dry climatic regimes generally have higher root to shoot ratios (R:S) and deeper root systems, than the species that are suited to moist climatic regimes (Pallardy and Rhoads, 1993). Such evidences has probably lead to the general concept that trees frequently respond to dry conditions by altering R:S ratios and by increasing rooting depth (Pallardy, 2010), and several hypothesis have arisen from this general concept concerning the effects of long-term decreases in water availability, like the increases in fine-root biomass and deeper penetration of roots to deeper layers of soil (Joslin et al., 2000).

3 Methodology

3.1 Study site:

The drought experiment is being carried out in two ecologically important forest types in Bhutan; Subalpine conifer forests and mixed broad-leaved forests, at two different altitudinal locations. One experimental site is Tashigang Gompa (TG) located at an altitude of 3290 metres above sea level (masl) in the cool conifer forests of Dochula pass in the Thimphu District. Another experimental site is at Pangsho Gompa (PG) located at an altitude of 2352masl in broadleaved forests of Nahi Gewog (Village), under Wangduephodrang District.

3.2 Site description

3.2.1 Site selection

For the experimental plots, four stands, two in the conifer zone and two in the broad-leaved zone, having typical structural characteristics and lying within the established long term monitoring plots were randomly selected. Sites having a representative tree composition, density and a minimum number of dead trees, and constant soil depth were considered. Sites having compacted soils, on steep slopes, shallow ground water and near existing footpaths and forest trails was excluded. Tree stands with bamboo understory was also excluded from the selection.

3.2.2 Tashigang Gompa (TG).

The forest of Tashigang Gompa (TG) is recognized as sub alpine coniferous forest at an altitude of 3290m. Mean annual temperature is 14°C and the precipitation is around 1427 mm per year. The forest soil found at this altitude are brown forest soils; the soil is brownish black, clay loam, moderate granular structure, moist and sticky, they consist of relatively thin dark porous 'A' horizons and brown colored 'B' horizons (Ohsawa, 1987). The forest is dominated by Hemlock (*Tsuga dumosa*). It is a forest of dense growth of small crooked trees or evergreen shrubs, *Rhododendron arboreum* being one of the most common species. The next dominant species is *Quercus semecarpifolia* (Oak). The other species found are *Acer campbelli*, *Pieris formosa*, *Picea spinulosa*, *Juniper species*, *Abies densa*, *Ilex dipyrena*, *Taxus baccata*, *Prunus spp.* *Osmanthus spp.*

3.2.3 Pangsho Gompa (PG)

The forest of Wangdue Phodrang District is classified as mixed broadleaved forest of warm temperate zone at an altitude of 2352m. The soils found in this forest are yellow brown forest soils, its characteristics are dark brown, silt loam, weak medium granular structure, moist and non-sticky (Ohsawa, 1987). They have been developed under the evergreen broad-leaved forests. The soils have brighter yellow colored 'B' horizon than those of Brown forest soils. The forest is characterised by dense canopy, with fairly large girth and medium height. Underneath the forest, undergrowth occurs. The mean annual temperature of this location is 22°C and precipitation is around 1000 mm per year. Dominant tree species are evergreen and deciduous oak (*Quercus lanata* and *Quercus griffithii*) and followed by *Rhododendron arboreum*. The other species found are *Lyonia ovalifolia*, *Symplocus lucida*, *Eurya acuminata*, *Symplocus sumunthia*, *Symplocus dryophila*, *Sorbus spp.*

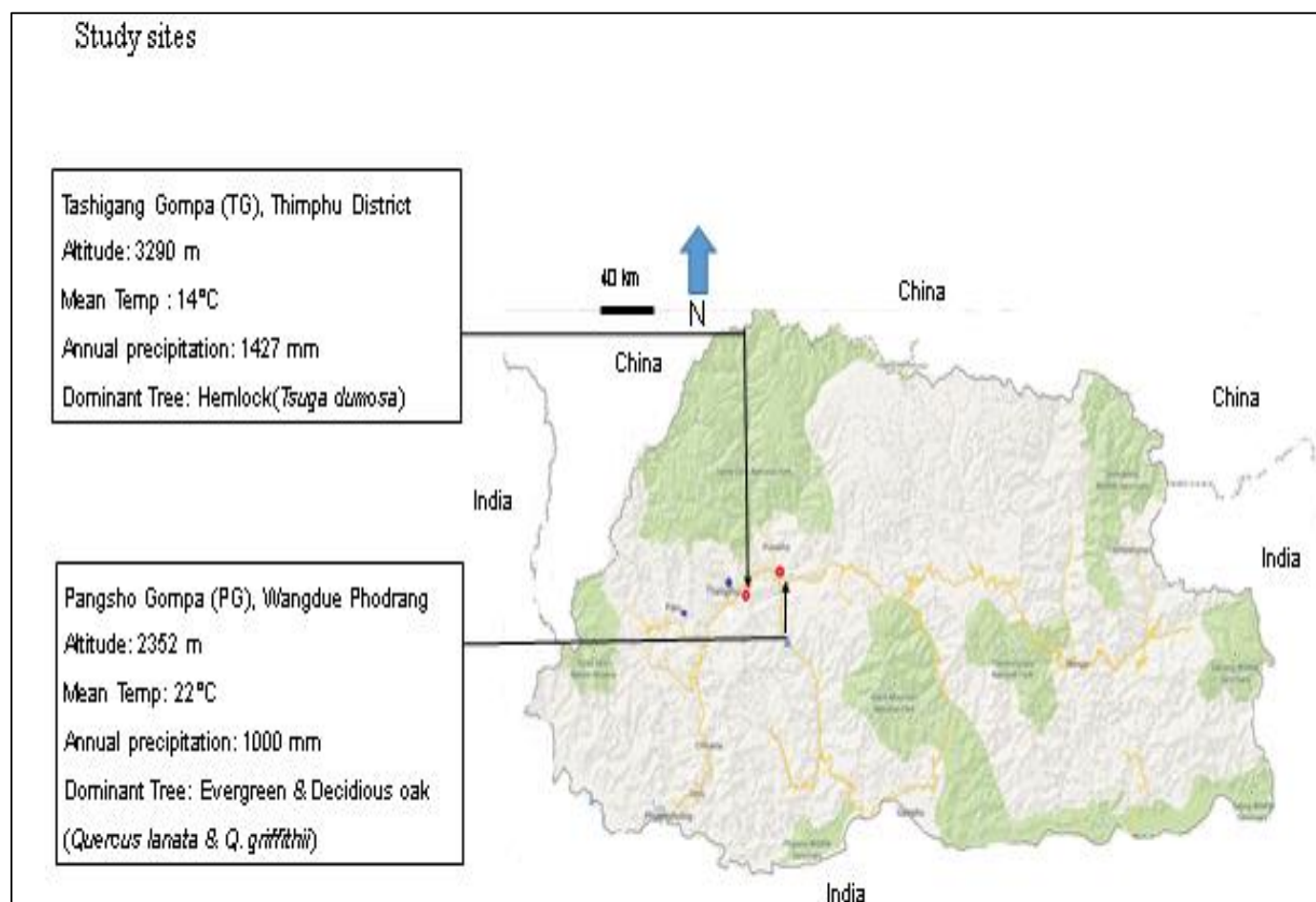


Figure 1: Map of Bhutan showing the location of two research sites.

3.3 Species Composition of the study sites

An inventory was carried out, in order to find the different species found in the forest. The detailed tree composition of both sites has been presented in Figure 2 below.

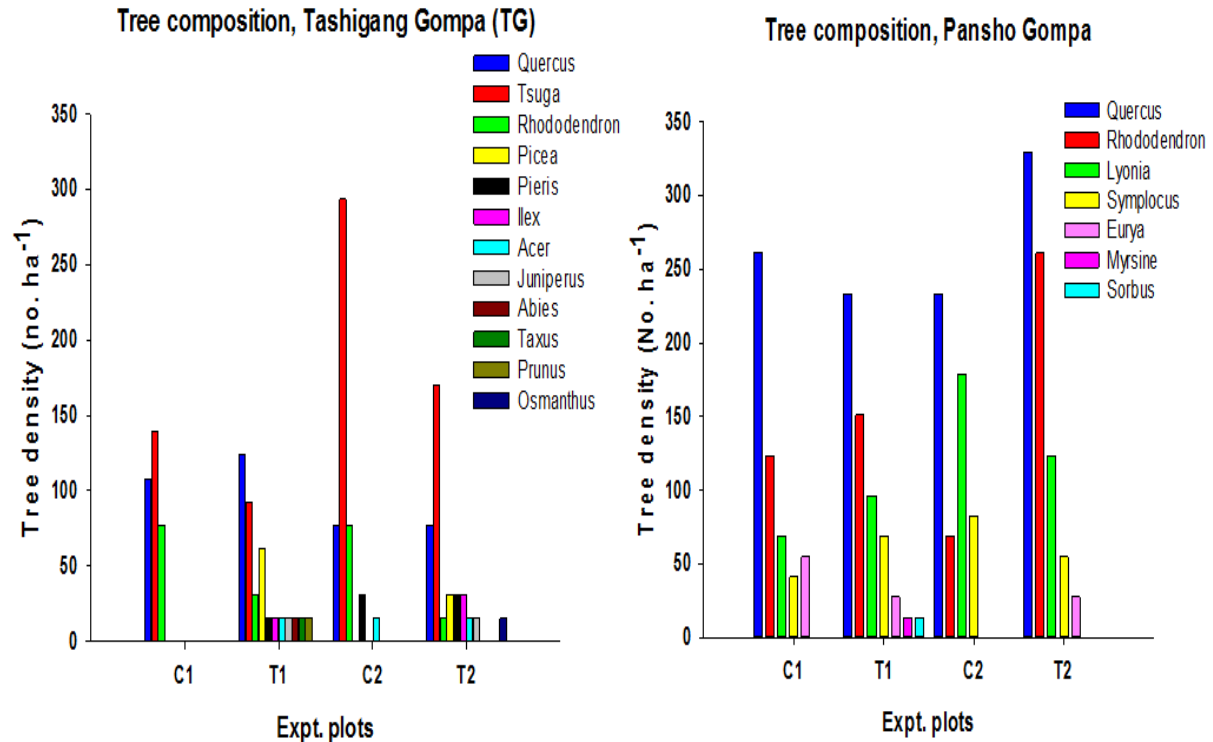


Figure 2: Tree density (expressed as number of tree per hectare) at different experimental plots of Tashigang Gampa (TG) and Pangsho Gampa (PG). C1 & C2 and T1 & T2 are control and treatment (roofing) plots respectively.

In Tashigang Gampa, the most dominant tree species is *Tsuga dumosa* followed by *Quercus semecarpifolia* (Oak). The third dominant species is *Rhododendron arboretum*. The other species found are *Acer campbelli*, *Pieris formosa*, *Picea spinulosa*, *Juniper species*, *Abies densa*, *Ilex dipyrena*, *Taxus baccata*, *Prunus spp.* *Osmanthus spp.*

In Pangsho Gampa, the most dominant tree species are evergreen and deciduous oak (*Quercus lanata* and *Quercus griffithii*) followed by *Rhododendron arboreum*. The other species found are *Lyonia ovalifolia*, *Symplocus lucida*, *Eurya acuminate*, *Symplocus sumunthia*, *Symplocus dryophila*, *Sorbus spp.*

3.4 Experimental Plot design

The experimental plot size was 25 m (towards slope direction) x 29 m (along contours). Four plots was established in the cool conifer forest (2 roof plots, 2 control plots), Tashigang

Gompa, Thimphu and four plots (2 roof plots, 2 control plots) in the warm broadleaved forests in Pangsho Gompa, Wangduephodrang.

Measurements were conducted in 20 subplots of 5m x 5m under the roof, with a 1m wide buffer at the bottom, and 3m wide buffer upslope of the plot, and 2.5m a buffer at two lateral margins. A total of 80 subplots of 5m x 5m in each experimental site were established (figure 3)

Plot Layout:

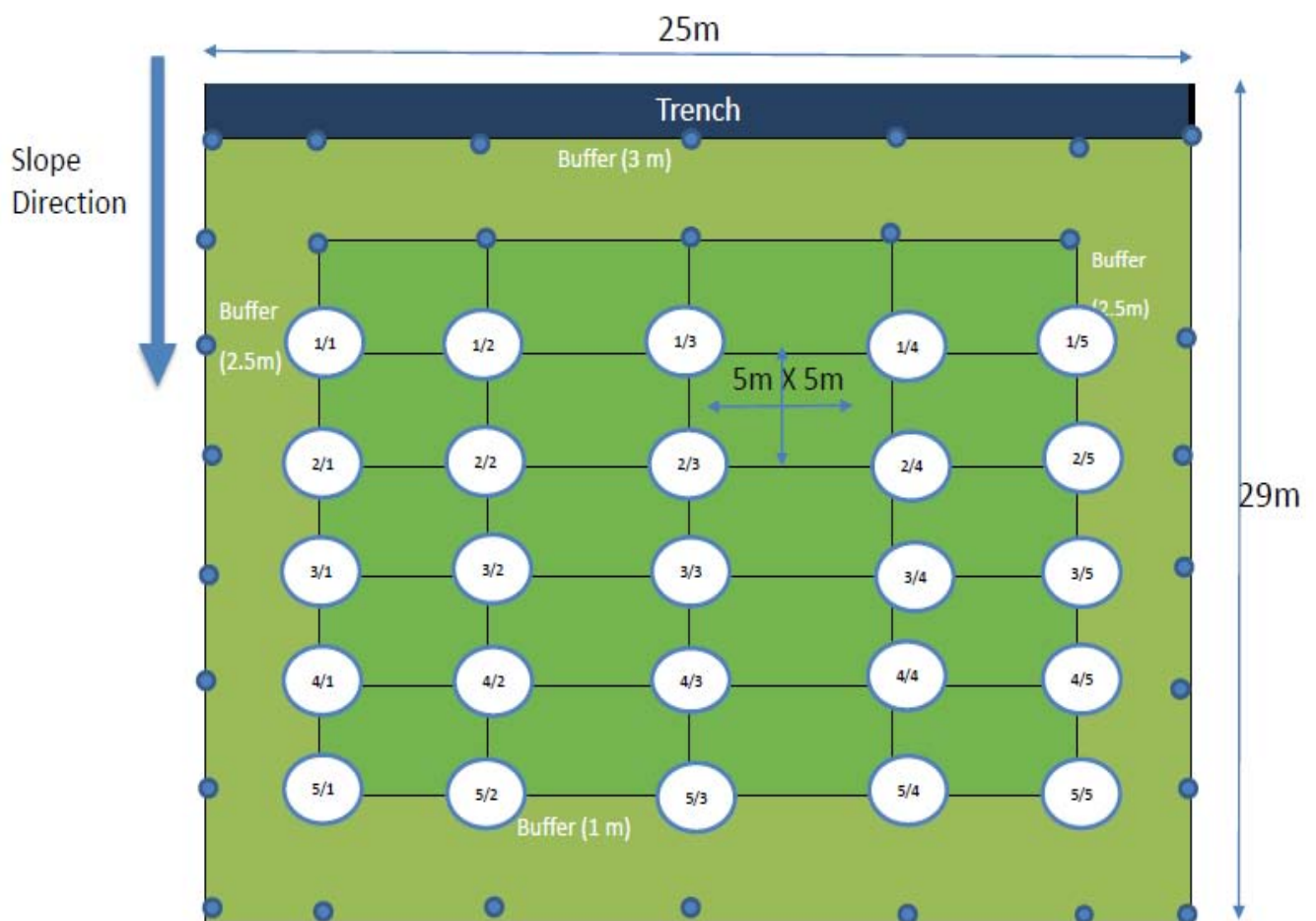


Figure 3: Detailed plot layout of 29m x 25m, showing the labelled subsections, and 20 subplots of 5m x 5m each.

3.5 Roof Construction

Roofs of dimension, 25 m (Horizontal) x 29 m (along slope) were constructed using bamboo poles and a transparent waterproof plastic foil. The roof was raised at a height of 1-2m above ground.



Photo 1: Construction of bamboo roofs up to 1-2m high from the ground.



Photo 2: Transparent plastic foil over the bamboo roof.

3.5.1 Trench

A deep trench was constructed above the plot. The trench reached the parent material. The trench was covered with bitumen felt, which was attached to wooden frame, and the wooden

frame was attached to the trench wall. In the ground of the trench, a drainage pipe was installed. This leads the water being drained out properly, without entering and disturbing the experimental plots during the rainy season.



Photo 3: Showing the trenches.

3.5.2 Pathway

A network of designated pathway to walk was made inside the plot using long ribbons as shown in the picture below. This was done in-order to keep disturbance to minimum, while carrying out the measurements and experiments.



Photo 4: Designated pathways for walking, made by coloured ribbons.

3.6 Roofing Periods

The roof was on from May, 2014. The roofs were kept for a period of four months (May-August), which is the monsoon season in Bhutan. The roof was then taken off in the month of August, 2014, towards the end of monsoon season. The first set of samples was taken just before the roof went on, that is in the beginning of May. The second sets of samples were collected after the roof was removed, in the end of August. Then the third and the last sample of the year were taken in the end of November, after the experimental plots were left without roofing, as a recovery period. Soil moisture content in control and roof plots, during the experimental period for both sites is given in figure 4 below.

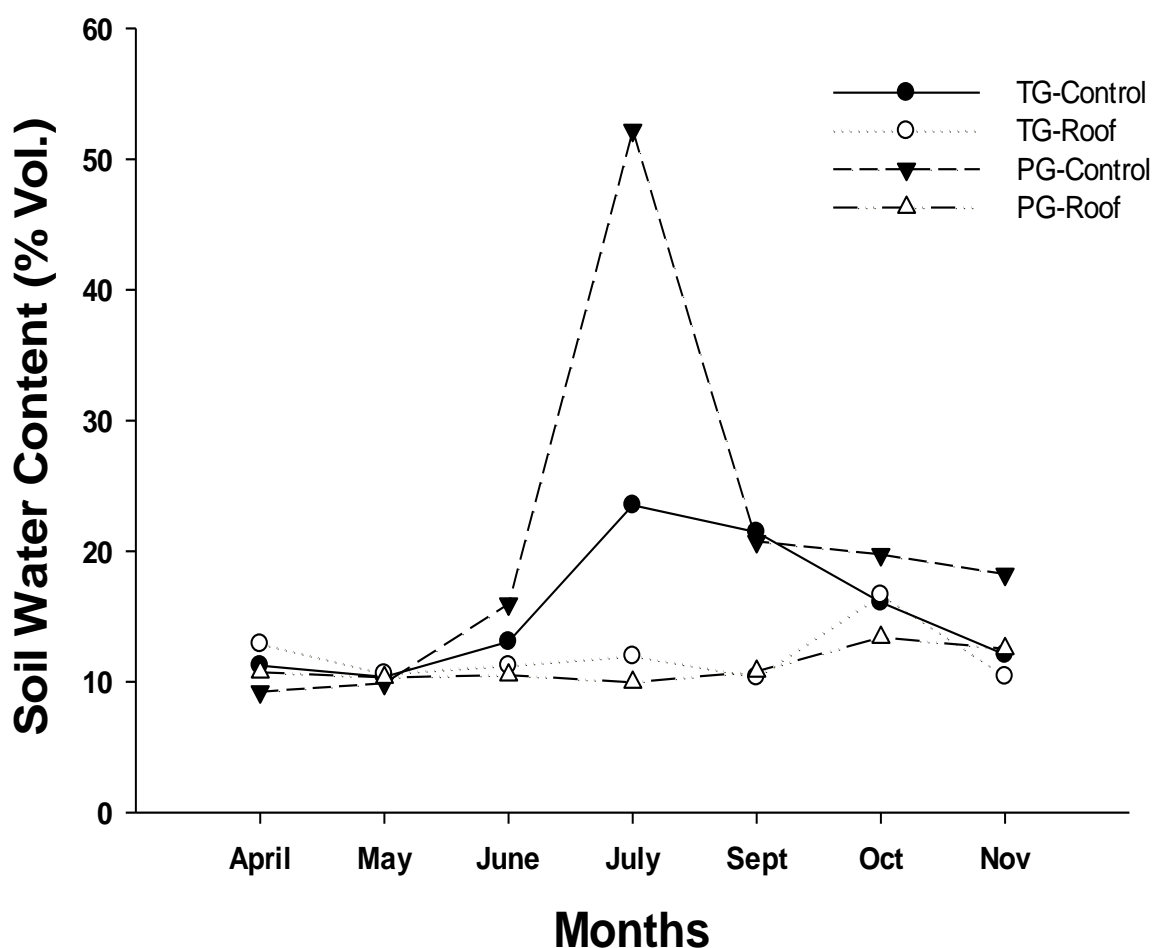


Figure 4: Soil water content (% Vol.) of control and roof experimental plots at Tashigang Gompa (TG) and Pangsho Gompa (PG) during experimental periods.

3.7 Estimation of below ground Biomass (Root study)

3.7.1 Sampling technique

The choice of a proper method for the root study is one of the crucial questions in the planning stage, as the Labour, time and external influence parameters set boundaries within a method which is chosen. Observation methods are only meaningful for a long-term study and hence not applicable. Indirect methods are costly and need special equipment. Consequently, only one group, the root system extraction methods, was left. Generally the impact of any study on the ecosystem should be as low as possible, hence in order to have as low disturbance and ecological impact to the environment, the decision was made in favour of the sequential soil coring method. This method is most suitable for taking volumetric soil and root samples, and is probably the most frequently used method of root sampling (Brunner et al., 2013). This method allows a repeated sampling of restricted experimental plot, and the sampling of plots with limited soil depth and difficult geography (Mackie-Dawson and Atkinson, 1991). The root system of the tree species is not very much damaged by taking such small core samples with this technique in comparison to the excavation methods (Vogt et al., 1998). In addition, since the method is less time consuming, and the equipment can easily be moved in the forest when working, the sequential coring method has been preferred in contrast to the other existing methods. The question of the inside diameter is a central one. According to Vogt et al. (1998), many research workers use core diameter of 7 cm, and a core diameter is seldom exceeds 10 cm, even when using mechanized sampling techniques. Soil cores which are collected with a 7 cm core diameter can vary in length relative to the undisturbed soil, depending on occurring soil parameters. The corer used for this study has diameter of 7.5 cm, and a length of 53.5cm. The model which has been used for this study is driven into the soil by using a damping head and a plastic hammer to avoid deformation of the coring tube.

3.7.2 Sequential Coring

Materials required: Stainless steel cylindrical corer (7.5cm diameter), steel rod, Styrofoam tray, plunger, knife, measuring tape, plastic hammer, and plastic bags.

Sequential coring method was used for the collection of soil and root samples. It was decided to collect the soil profiles till 30cm depth.

At the least two people were needed for the field work. The cylindrical corer was kept upright on the floor, and then the top was covered with the cylinder cap. The hammer was hit on the

cap, till the cylinder was 30 to 40 cm below ground. Care should be taken to ensure that the edges of the cylinder are well sharpened, because a blunt edge does not cut roots, but rather drags them along the wall of the corer as it penetrates the soil, and this might cause an underestimation of the results (Polomski et al., 2002). After the cylinder corer was pulled up all the way, the cylinder corer filled with soil was placed on the edge of the Styrofoam tray, and the plunger was inserted from behind the cylinder, till all of the soil was laid out in the Styrofoam tray. The soil profile was measured using measuring tape and the extracted core samples were divided into three depth sections of 0-10 cm, 10-20 cm and 20-30cm soil layers. Every 10cm of soil from the soil profile was cut with sharp knife and each 10cm core was packed separately in the labelled plastic bags respectively. The knife used should be sharp, otherwise the blade will not cut the roots and, as a consequence, it will drag the roots along the intersection, and this would make it very difficult to assign the root (or parts of it) to either the left or the right side of the intersection.



Photo 5: Materials needed for sequential coring; Steel corer, steel rod, Styrofoam tray, plunger, plastic hammer and bag.



Photo 6: Sequential coring till 30cm depth.

3.7.3 Sampling Design

Systematic random sampling was followed while collecting the samples. In order to standardize the number of sample replicates taken, we took into consideration the uniformity of the slope and the species distribution in the area. We came to the conclusion of having six replicate sampling in each research plot. During the first sampling in May, ten replicate samples were collected from subplots of each plot. During the second sampling interval in August, six replicate sampling were taken from subplots of each plot, and similar trend was followed in the third sampling period in November. This was carried out in all of the eight plots of two research sites of Pangsho Gompa and Tashigang Gompa.

The first sampling was carried out between 17th of April to 5th of May, 2014. Ten replicate samples were collected from subsections of each research plot, as shown in the figure 5 below.

10 cores from each plot with 0-10, 10-20 & 20-30 cm depths

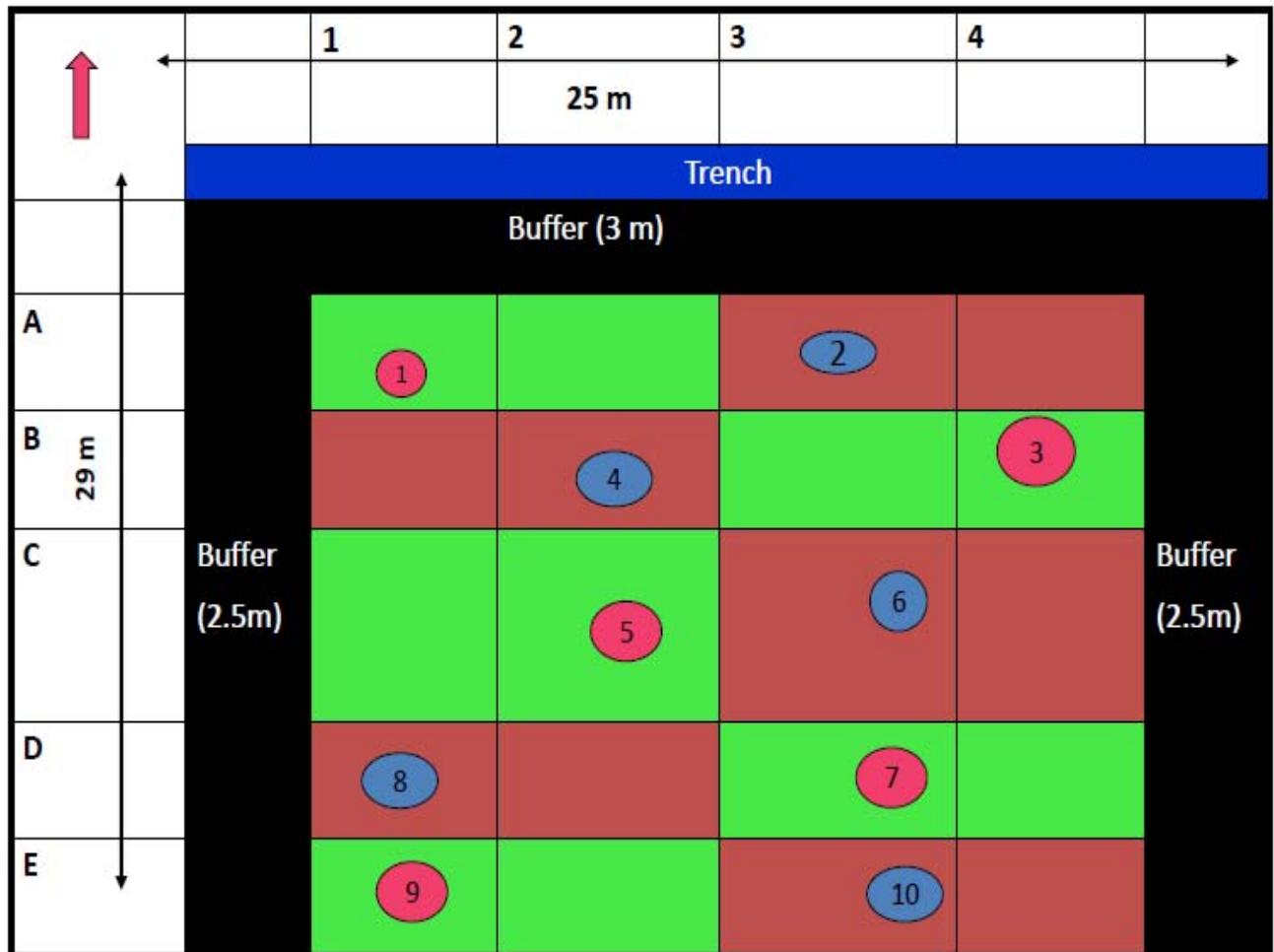


Figure 5: Showing the 10 replicate samples taken from each research plot in the month of May.

The second samplings were carried out from 21st of August, 2014, and the third samplings were carried out from the 15th of November, 2014. In both the sampling dates, six replicate samples were collected from subsections of each research plot, as shown in the figure 6 below.

6 cores from each plot with 0-10, 10-20 & 20-30 cm depths

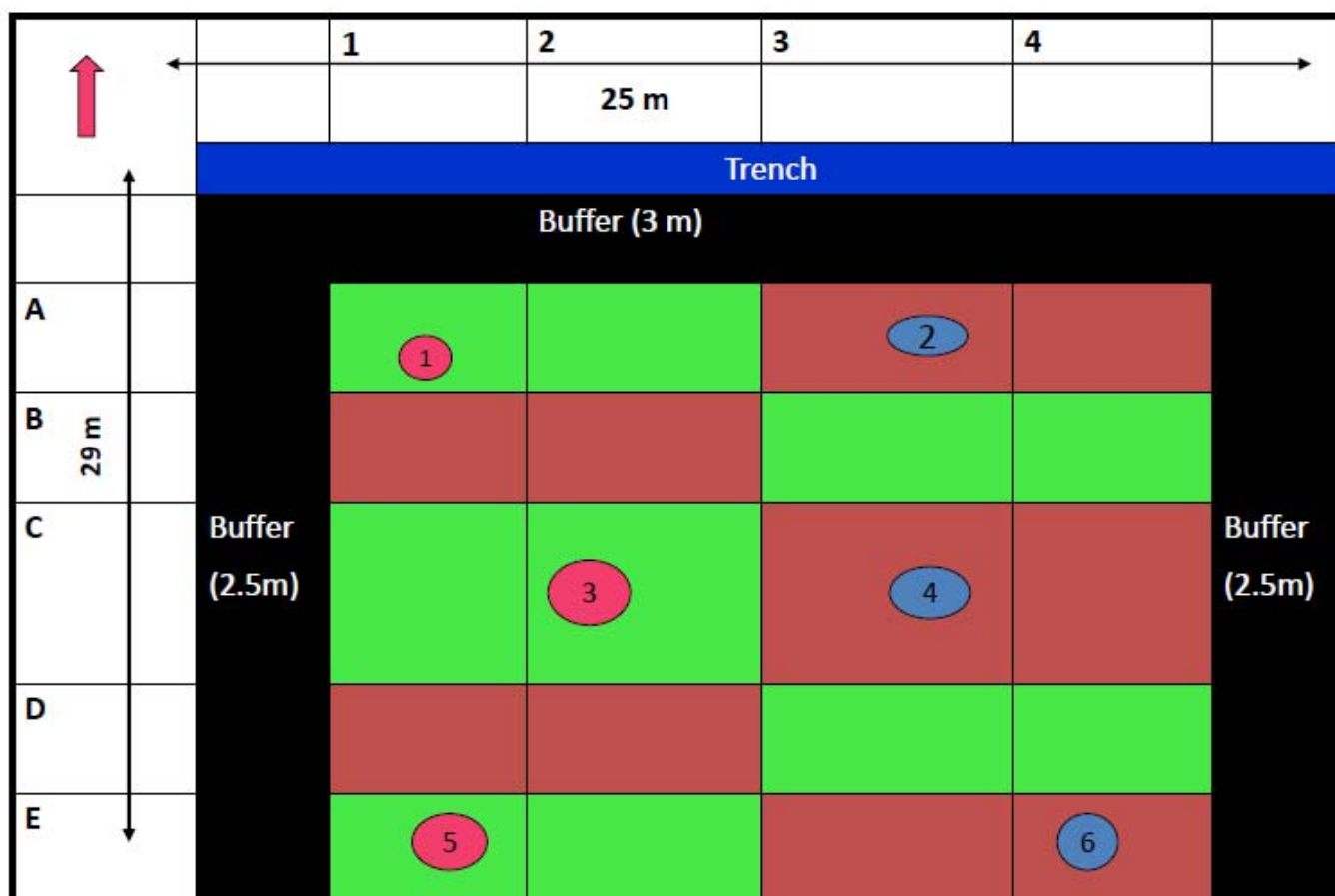


Figure 6: Showing the 6 replicate samples taken from each research plot in the month of August and November.

3.8 Sorting Root from Soil

The soil samples were taken out of the plastic bags and dried for a day. This is in order for the soil to dry, thus making it easier to separate roots from the soil. After drying it, the soil from each profile was laid on the table, and respectively the roots were separated from the soil (Photo 7). The separated roots from each of the profile was packed in the sample bags and labelled accordingly. All of the root samples were packed in one unit and was brought to

University of Natural Resources and Applied Life Sciences (BOKU), Vienna for further analysis.



Photo 7: On the left; soil samples being dried before sorting the roots. On the Right; sorting of roots from soil.

3.9 Laboratory Work

3.9.1 Fine root, Coarse root and Necromass sorting

The relative time and labour needed for taking the core samples in the field are much less, compared with the additional work of washing them, separating the roots by species and sizes, and measuring the necessary root parameters (Bohm, 1979). About one quarter of the processing time was spent for taking the core, and three quarters for removing the soil and separating the roots by sizes and by species.

The root samples were stored in the cold freezer before the commencing of the laboratory work. Root samples from each of the profile were taken serially and were taken for sorting. Roots from each of the sample bags were put into a large transparent bowl filled with tap water. The roots were then washed free of soils and other materials attached. After cleansing the roots, the murky water was drained out of bowl and new batch of clean water was filled in the bowl. The roots were ready for sorting in size class and species class.

Rytter (1999) classifies organic material as coarse roots (diameter > 2 mm) and fine roots (diameter ≤ 2 mm). Following these recommendations, the same definition for coarse and

fine roots has been used for this study. The size sorting was done in the following manner. The roots were divided into three size classes based on diameter. The root measurement of diameter less than 2mm were taken as fine roots. The roots of diameter between 2mm to 5mm were taken as coarse roots. And the roots larger than 5mm were not counted in the samples in-order to avoid large deviation in the result. The dead roots were taken as the necromass. A root was determined as dead if the colour was dark brown and if it was without finer ramifications and could easily be broken. The same determination criterions were used by Rytter (1999). The fine roots, coarse roots and necromass were separated and packed in the small paper bags, which are necessary for drying the samples in the oven. The paper bags were labelled accordingly. The label included information about the treatments, root size, soil depth, tree species, sample number, and location of the sample plot. This makes it possible to assign the sample at a later stage.



Photo 8: laboratory work equipment: scissor, scale, magnifying lens, forceps, and water bowl.



Photo 9: Laboratory work on sorting of fine-root, coarse root and necromass.

3.9.2 Sorting by Species

There were three dominant tree species to be sorted and investigated in total. One was *Tsuga dumosa* (Hemlock) from Tashigang Gumpa. Two species to be sorted from Pangsho Gumpa were Oak (*Quercus lanata* and *Quercus griffithii*) and *Rhododendron arboretum*. The roots were identified with the distinctive features with the aid of a magnifying glass. Different root species were sorted looking at the; 1) coloration of the root, 2) branching pattern of the fine roots 3) change in coloration when scratched underneath the root surface, 4) smoothness of the root cover.

3.9.2.1 *Tsuga dumosa* / Hemlock

Tsuga dumosa roots are light red in colour and has a rough bark compared to *Rhododendron arboretum*. When scratched underneath, it has a light red film and if further scratched, it has white coloration. The branching patterns of fine roots form a network



Photo 10: The left picture shows the fine root of *Tsuga dumosa*, light red in colour. The right diagram shows the underneath red coloration, when the root cover is scratched.

3.9.2.2 *Rhododendron arboreum*

The *Rhododendron arboreum* roots have dark reddish brown coloration. If scratched underneath, it has a white coloration. The branching patterns of the fine roots are in wavy fashion. The root cover is smooth compared to *Tsuga dumosa*.



Photo 11: The left picture shows the fine root of *Rhododendron arboreum*, reddish brown in colour. The right picture shows the white underneath coloration when the root cover is scratched.

3.9.2.3 *Quercus Spp.* / Oak

The *Quercus spp.* roots have light brown coloration, and when scratched underneath, it has dark brown coloration. The root cover is rough and the branching patterns form a network. We could not separate the two *Quercus spp.* (*Quercus lanata* and *Quercus griffithii*), as their roots had similar characteristic which made it difficult for identification, while sorting. Therefore we took it as one sample.



Photo 12: The left picture shows the brown colour of *Quercus spp.* fine roots. The right picture shows the dark brown underneath coloration of the root, when scratched.

After the sorting of the roots into its fine, coarse and necromass component of each species, the packed samples were taken for oven drying to remove any moisture present in the roots. According to Vogt et al. (1998), the drying could be done at 60°C to 75°C, which will take longer time, but can have the advantage that this lower temperature prevents roots from being pulverized. Following this, all the samples were dried in the oven at 70°C for 48 hours. The samples were then weighed on a balance to four decimal points. The weight of the all the respective samples were recorded in the Microsoft excel sheet in the lap top.



Photo 13: Root samples dried in the oven at 70⁰C for 48 hours.



Photo 14: Left; weighing machine till 4 decimal points. Right; recording of sample weights.

3.10 Result Analysis

The recorded weight in grams of the samples was done in excel sheet. The mean, standard deviation and standard error were calculated. Further the samples were statistically analysed for ANOVA, using SPSS statistic software, and graphs were plotted using Sigma Plot software.

Microsoft excel sheet:

The recorded weight in grams of the samples was converted into gram per metre square, using the area of the cylindrical core.

Area of cylindrical core = πr^2 (where 'r' is the radius of the cylinder core)

After the conversion of all the data into gram per metre square, they were arranged in following order:

- i) Firstly for 0-30cm depth, the total biomass of fine root, coarse root and necromass was calculated, and then the same was carried out for each of the dominant species of Tashigang Gompa and Pangsho Gompa.
- ii) Secondly, soil depth-wise sorting and calculation of 0-10cm, 10-20cm, and 20-30cm was done separately, for each of the dominant species in both the sites. The same was carried out for the total fine roots and necromass.

Graphs plotted using Sigma Plot:

Line graph was plotted for the 0-30cm depth for the two forest types in Pangsho Gompa and Tashigang Gompa. The line graph showed the change in amount of root biomass production across the three experimental periods of; 1) Before roofing in May, 2) After roofing in August and, 3) After recovery period in November, for both the control and drought treatment plots.

Second set of graph plotted was horizontal bar graph; it showed the change in amount of root biomass with change in soil depth, in each of the dominant species across the three experimental periods, for the drought and control sites.

Statistical Analysis:

Statistical software SPSS programme was used to do the statistical analysis for the data acquired. One-way ANOVA was carried out for the 0-30cm depth, comparing the means of root biomass across the three experimental periods, for each of the dominant species in the control and roof plots of both the forest types. Post Hoc Tukey test was also carried out in order to see, if there is any significant seasonal variation of the fine root biomass. T- test was carried out for comparing the significant difference between the control and drought treatment plots in each of the three sampling intervals. The outcomes of the ANOVA was reported by presenting the probability (*P* values) of the null hypothesis being true at a significance level $\alpha = 0.05$

Secondly, two ways ANOVA was carried out for the depth wise distribution of the root biomass. Two independent variables are depth and treatment, and one dependent variable is root biomass (Depth x Treatment x Biomass). Post Hoc Tukey test was carried out for comparison between the depth wise distributions. The outcomes of the ANOVA was reported by presenting the probability (P values) of the null hypothesis being true at a significance level $\alpha = 0.05$

4 RESULTS:

4.1 Tashigang Gompa, root biomass, Total 0-30cm

Fine root ($\leq 2\text{mm}$) biomass in Tashigang Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 7. The higher fine root biomass was recorded in the control plots ($466.2 \text{ g m}^{-2} \pm 56.4$) than the treatment ($369.8 \text{ g m}^{-2} \pm 13.8$) in the month of May. However, this variation was not statistically significant ($P= 0.157$). Similar trend was also observed in August and November.

The seasonal variation in fine root biomass in both control and treatment follows the trend: May > August > November, but the seasonal variation was also statistically not significant (Fig. 7).

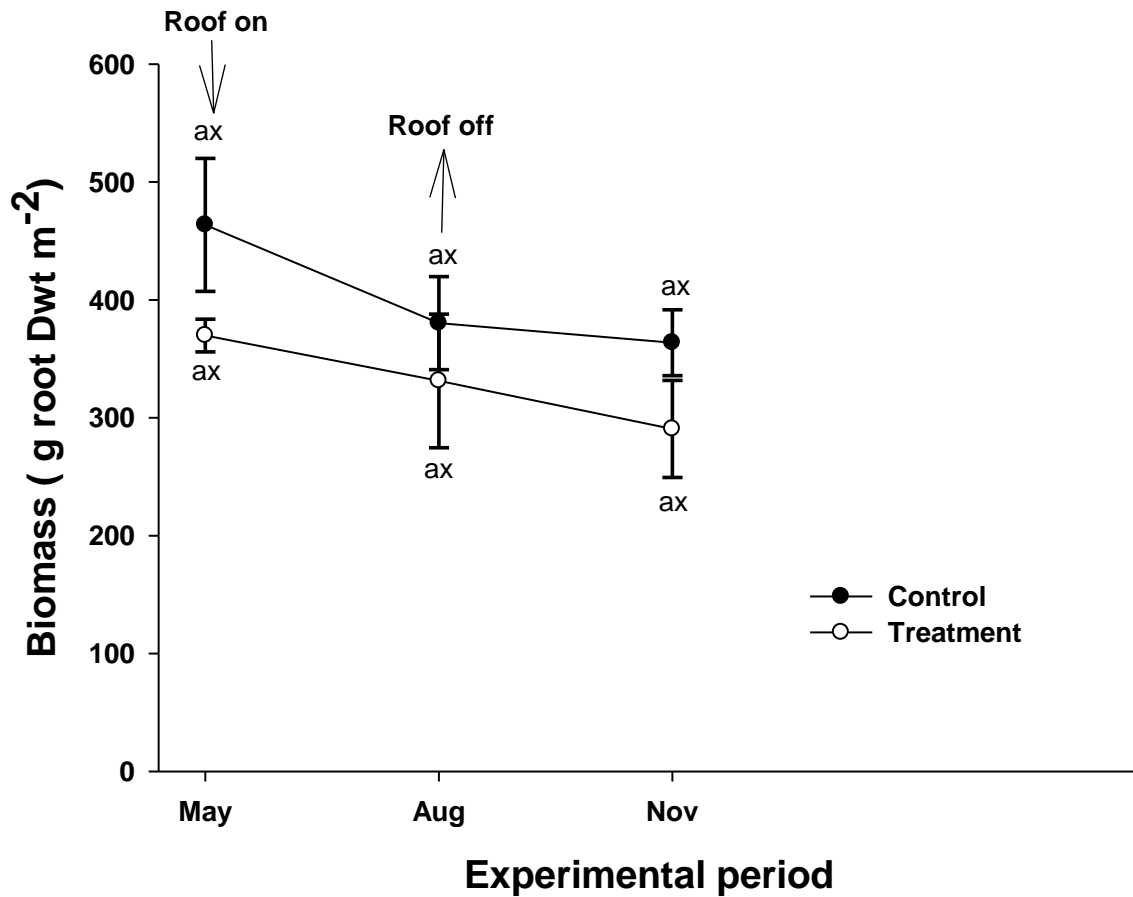


Figure 7: Total fine root biomass ($\leq 2\text{mm}$) in 0-30cm soil depth at Tashigang Gompa, in control and drought treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

Coarse root (2-5mm) biomass in Tashigang Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 8. The higher coarse root was recorded in control plots ($479.6 \text{ g m}^{-2} \pm 45.1$) than the treatment ($365.3 \text{ g m}^{-2} \pm 33.8$) in the month of May; However, this variation is not statistically significant ($P= 0.089$). Similar trend is also observed in August and November. The seasonal variation in coarse root biomass in both control and treatment follows the trend: May > August > November. The seasonal variation was statistically highly significant between May and August and also between May and November in control plots. In treatment plots, there was significant difference between May and November with P value of 0.04 (Fig. 8).

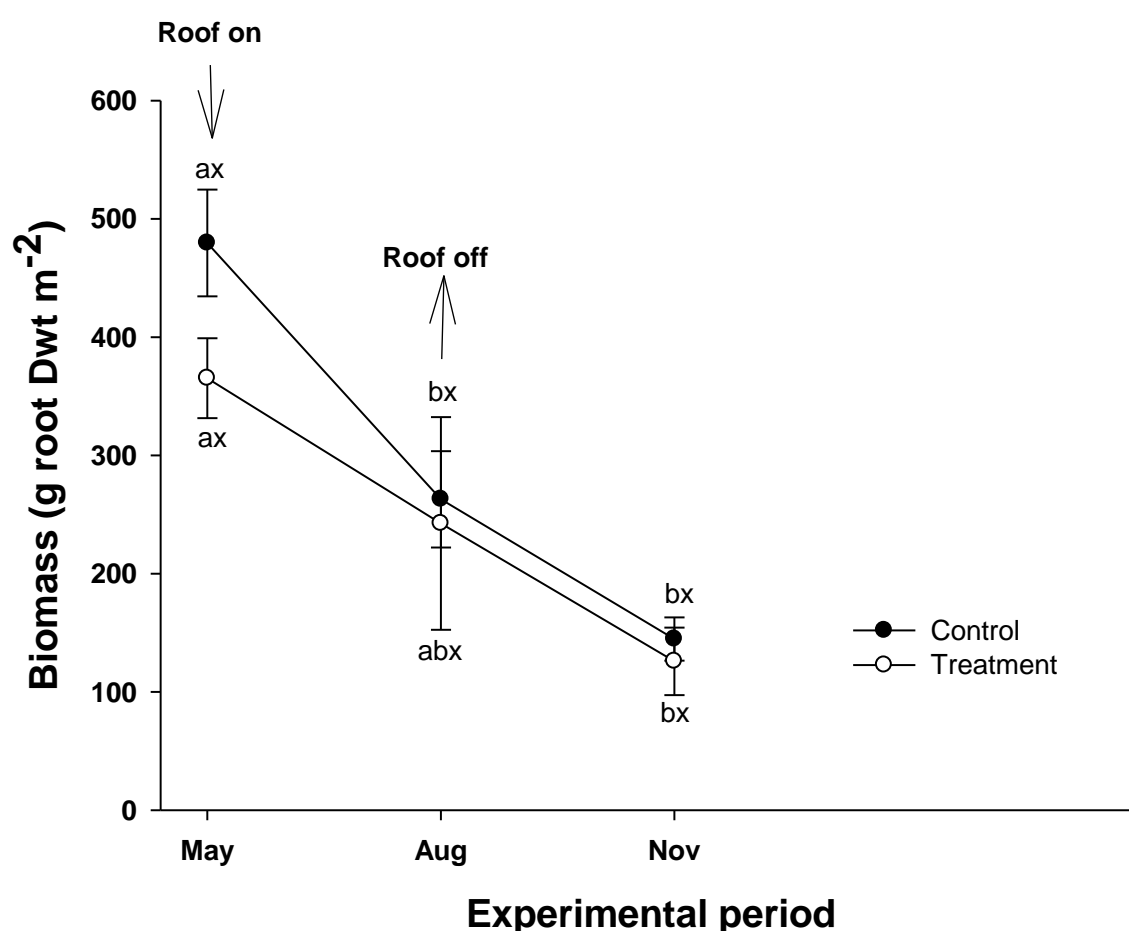


Figure 8: Total coarse root (2-5mm) biomass in 0-30cm soil depth at Tashigang Gompa, in control and drought treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

Necromass in Tashigang Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 9. No significant difference was shown between the control and drought treatment plots, across any of the three sampling dates.

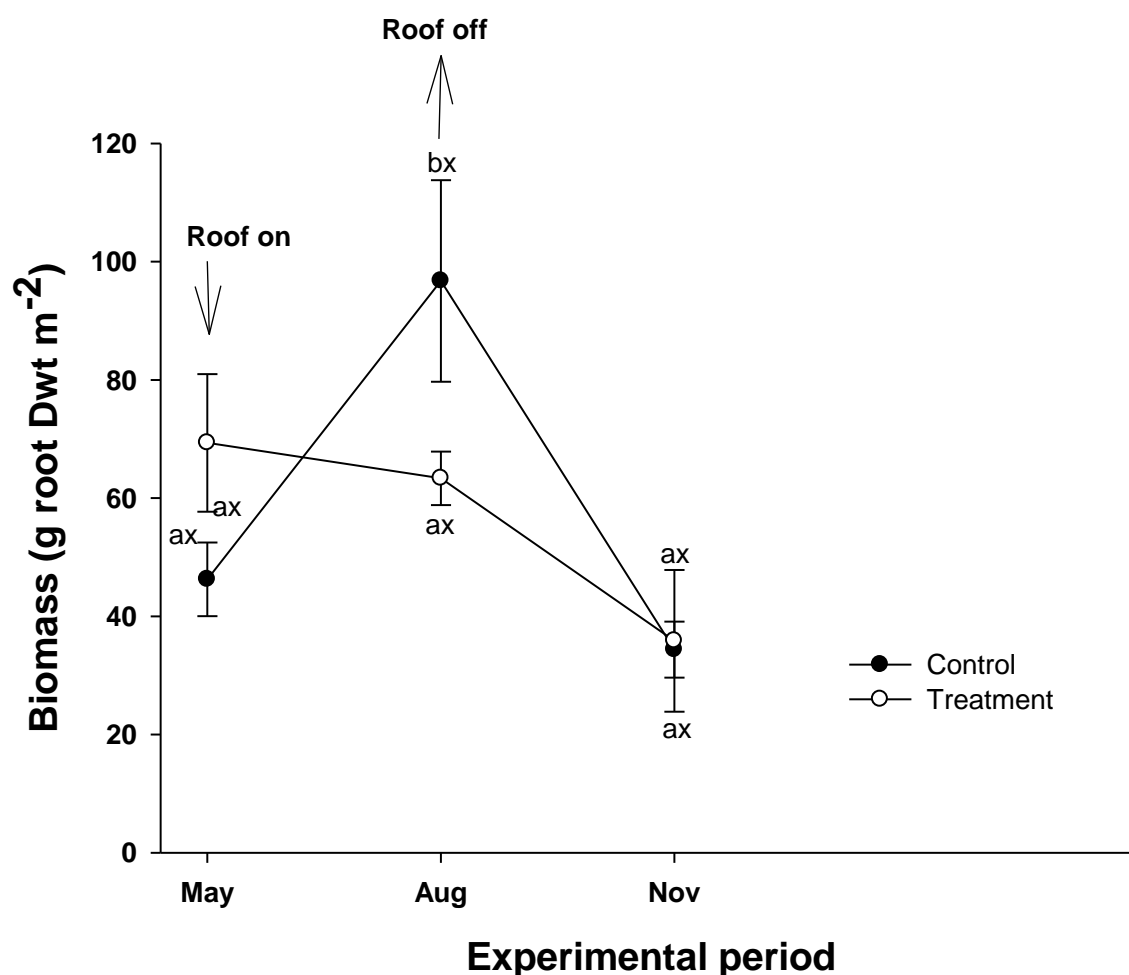


Figure 9: Total necromass in 0-30cm soil depth at Tashigang Gompa, in control and drought treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

The seasonal variation in necromass in control plots follows the trend: May < August > November. There was a significant increase in the necromass in the August month compared to May month ($P = 0.023$), and then it was followed by a significant decrease in November ($P = 0.007$). The seasonal variation in treatment plots follows the trend: May > August > November. No significant difference was found in the treatment (Fig. 9).

4.2 Tashigang Gompa, fine root biomass of dominant Species, 0-30cm

Tsuga dumosa fine roots biomass in Tashigang Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 10. The higher fine root biomass was recorded in the control plots ($233.8 \text{ g m}^{-2} \pm 39.9$) than the treatment (194.2 ± 11.4) in May. However, this variation was not statistically significant ($P= 0.37$). A similar trend was also observed in August, but in November, the treatment plot had higher fine root biomass than control plots, however this was not significant.

The seasonal variation in *Tsuga dumosa* fine root biomass in control follows the trend: May > August > November. And for the treatment plots: May > August < November. There was no statistical significant difference in any seasonal variations (Fig. 10).

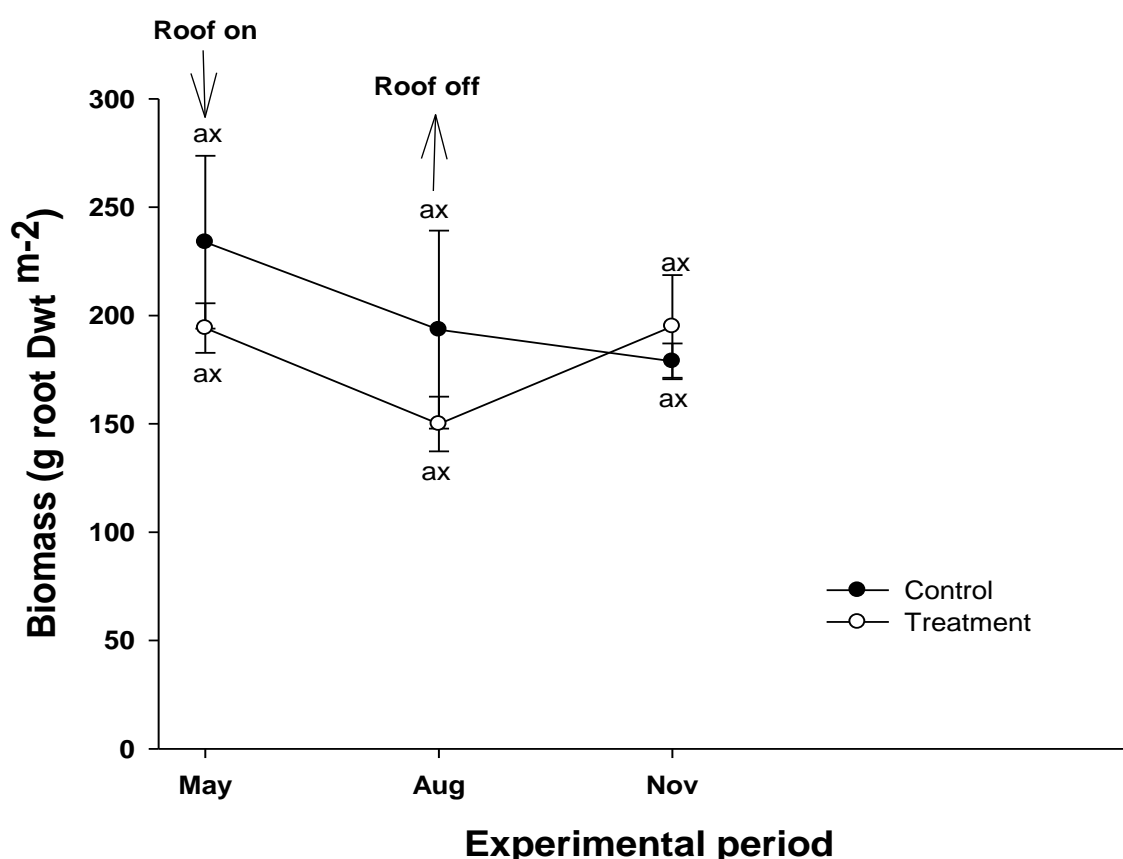


Figure 10: *Tsuga dumosa* fine root biomass in 0-30cm soil depth at Tashigang Gompa, in control and drought treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

4.3 Tashigang Gompa, Vertical distribution of root biomass

Fine root biomass:

There was a significant difference in the vertical distribution of the fine root biomass across different soil depths ($P=0.02$), with higher fine root biomass occurring in the 1-10cm soil depth and least in the 20-30cm soil depth. There was no significant effect of the treatments on the fine roots biomass (Fig. 11a)

During August, there was no significant effect of the soil depth nor the treatments on the fine root biomass at the Tashigang Gompa (Fig. 11b)

In November, there was a significant effect of the soil depth and treatments on the fine root biomass. The fine root biomass decreased with increasing depth at a highly significant level. The control plots had a significant higher biomass than the roof treatment with the P value of 0.046 (Fig. 11c)

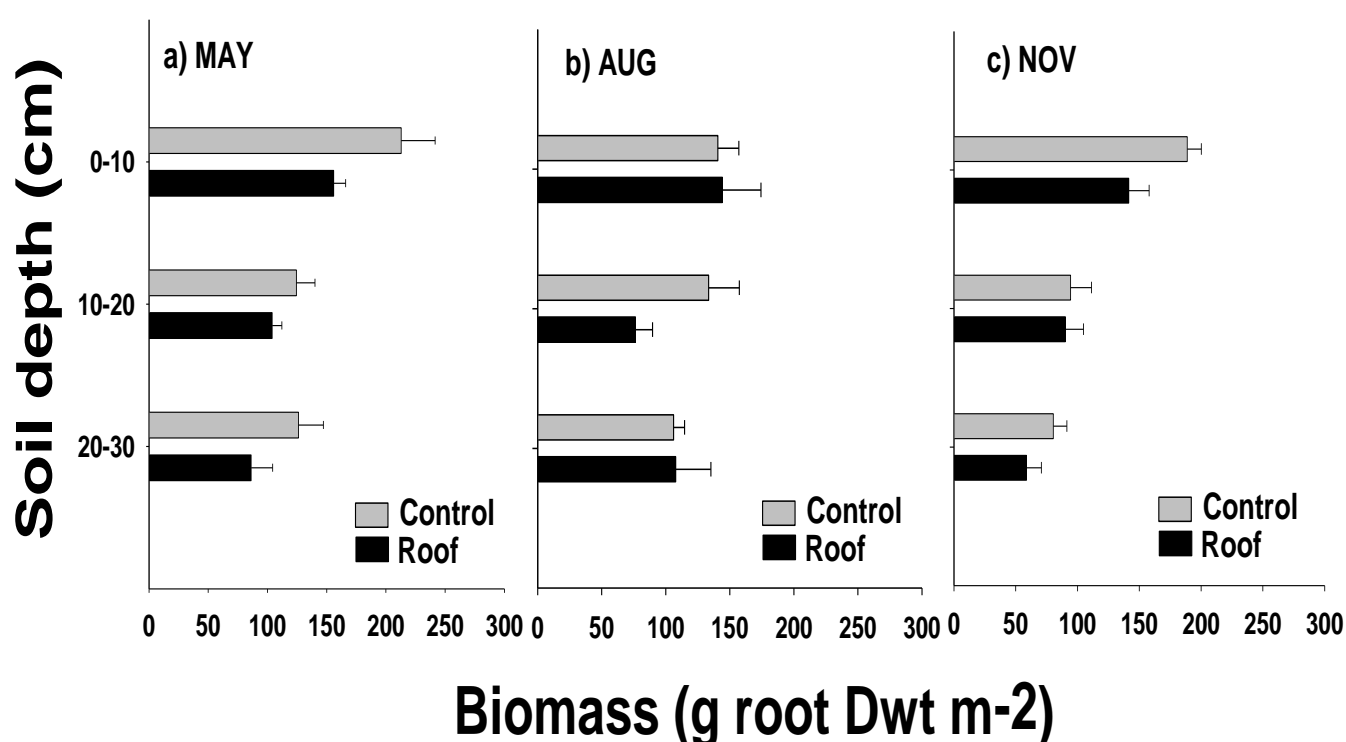


Figure 11: Depth wise distribution of fine root biomass at Tashigang Gompa, in control and roof plots across three sampling dates.

Necromass:

There was no significant effect by soil depth or treatments on the necromass at Tashigang Gompa, in May, August and November (Fig. 12).

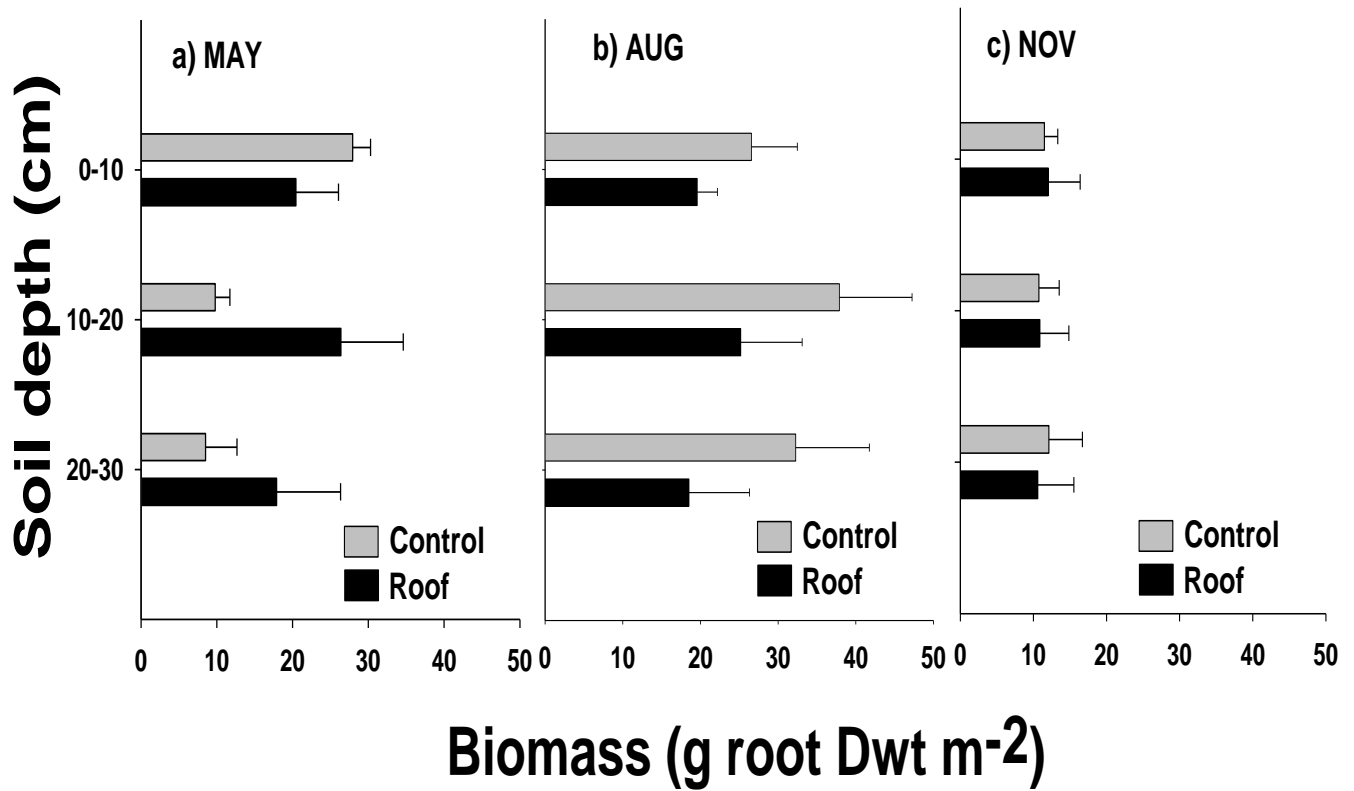


Figure 12: Depthwise distribution of necromass at Tashigang Gompa, in control and roof plots across three sampling dates.

4.4 Tashigang Gompa Species, Vertical distribution of fine root biomass

Tsuga dumosa

In May, there was significant decrease of *Tsuga dumosa* fine root biomass with increase in depth ($P= 0.006$), except between the depth of 10-20 and 20-30cm depth. And no significant effect of the treatment on root biomass was shown (Fig. 13a)

In August, no significant effect of the treatment and of soil-depth, was observed on the *Tsuga dumosa* fine root biomass (Fig. 13b)

In November, a significant effect of the soil depth on root biomass was observed. Fine root biomass decreased with increase in soil depth ($P= 0.002$) but no significant effect of the treatment was shown (Fig. 13c).

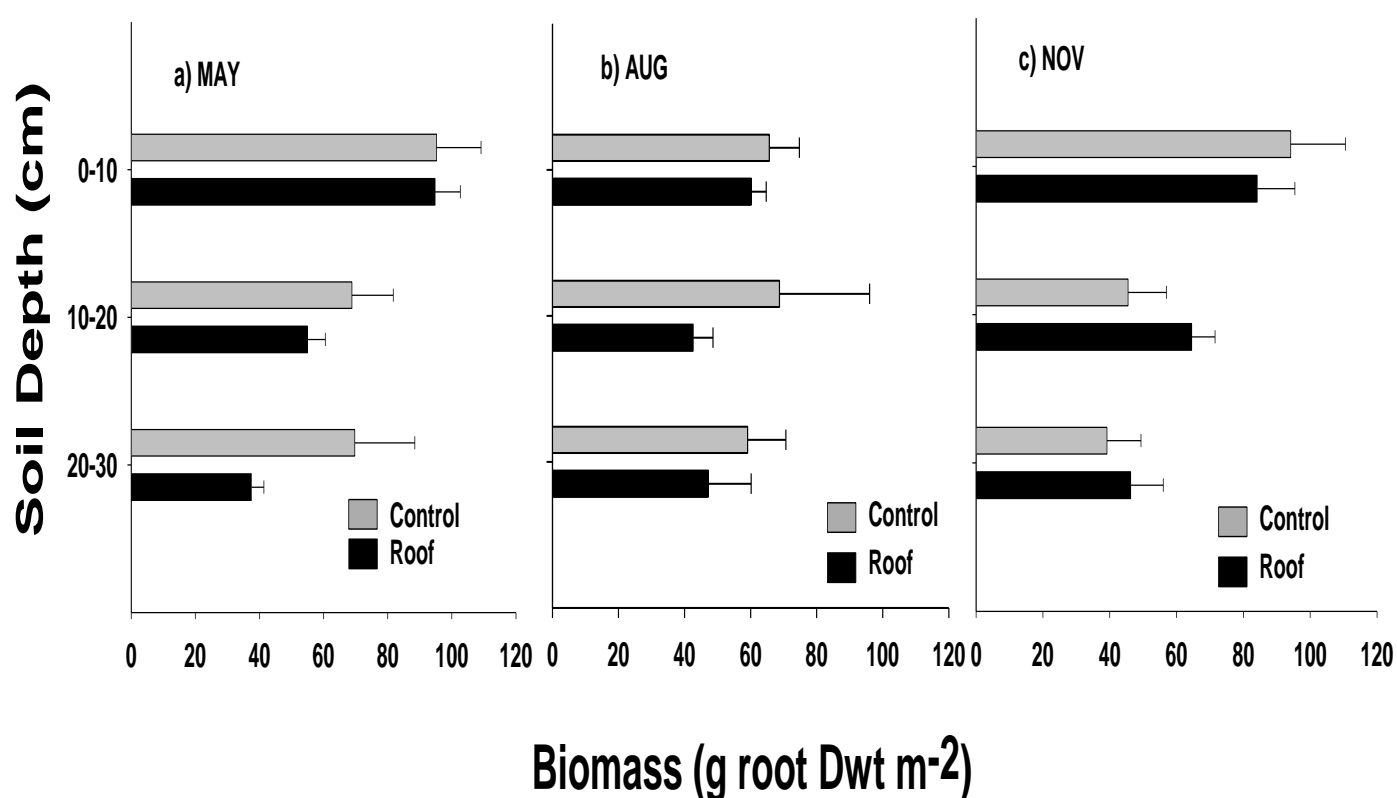


Figure 13: Depth wise distribution of *Tsuga dumosa* fine root biomass at Tashigang Gompa, in control and roof plots in three sampling dates.

4.5 Pangsho Gompa root biomass, Total 0-30cm

Fine root biomass in Pangsho Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 14. There was no significant difference between the control and treatment plots across any of the sampling dates.

The seasonal variation in fine root biomass in both control and treatment follows the trend: May > August < November. The seasonal variation was also statistically not significant (Fig. 14).

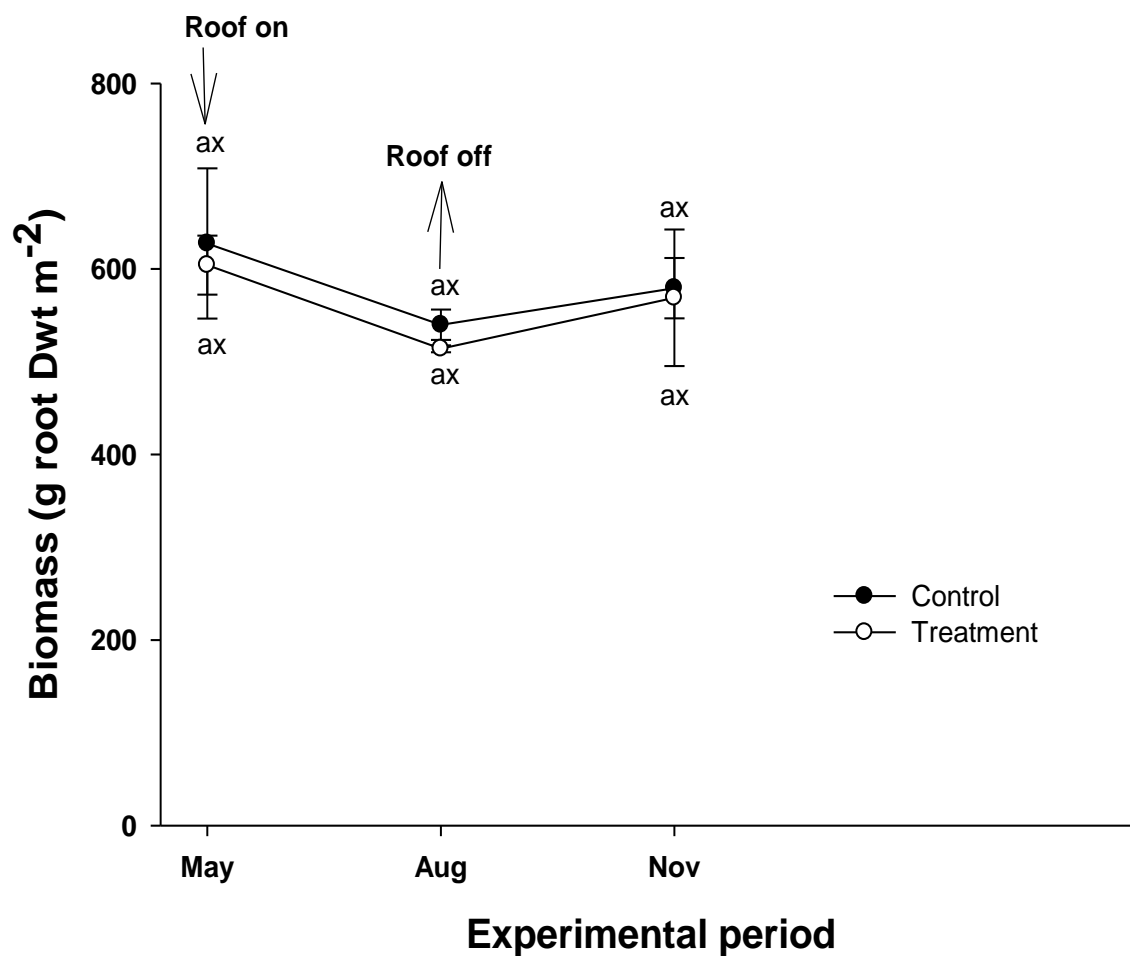


Figure 14: Total fine root biomass in 0-30cm depth at Pangsho Gompa, in control and drought treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

Coarse root biomass in Pangsho Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 15. There was no significant difference between the control and treatment plots across any of the sampling dates.

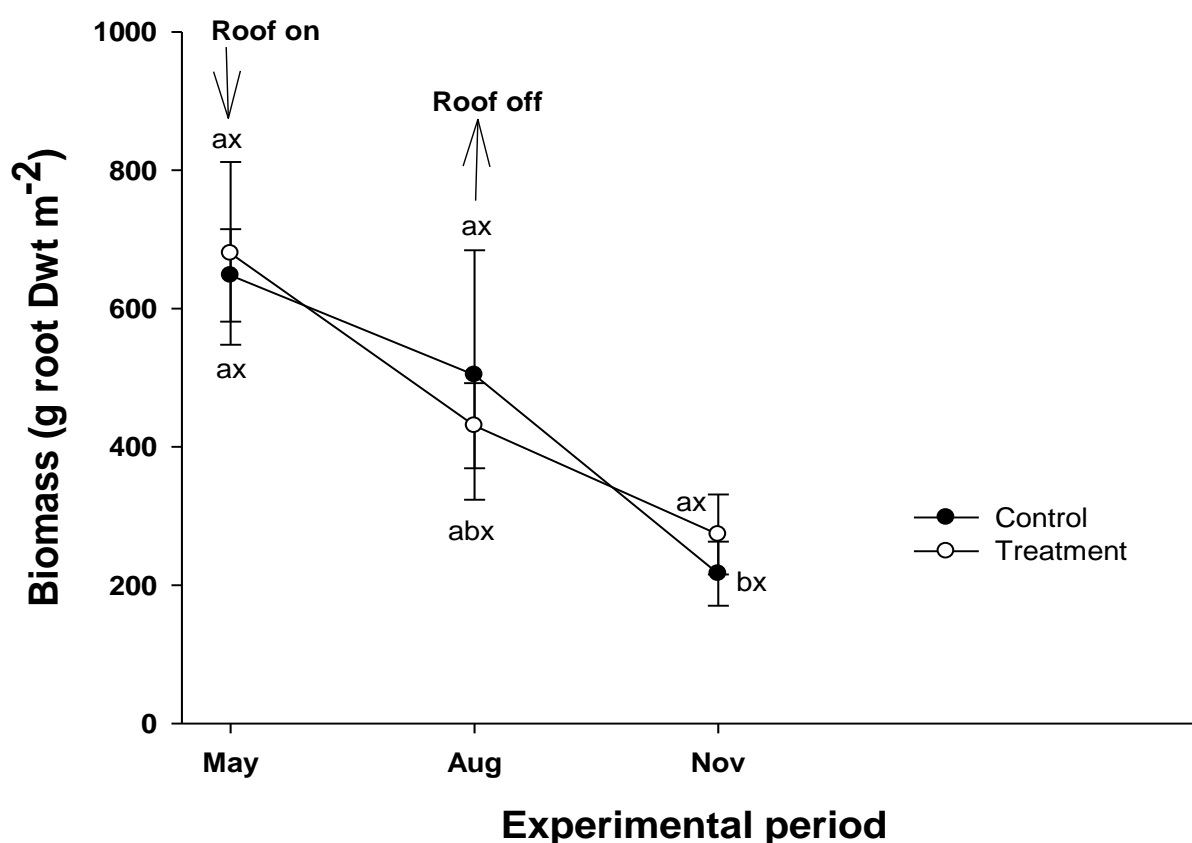


Figure 15: Total coarse root (2-5mm) biomass in 0-30cm soil depth at Pangsho Gompa in control and treatment plots across three sampling dates. Data points between sampling data (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

The seasonal variation in coarse root biomass in control plots follows the trend: May > August > November. There was no significant difference in seasonal variation of control plots. The seasonal variation in treatment plots follows the trend: May > August > November. There was a significant decrease in the coarse roots in the November compared to May ($P = 0.028$) in treatment plots (Fig. 15).

Necromass in Pangsho Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 15. In May, the necromass is higher in the control plots than the treatment, but in August, the treatment plots has higher necromass than the control plots, No significant difference was shown between the control and drought treatment plots in May and August. However significant difference was shown between the control and treatment plots ($P= 0.035$) in the month of November, with control plots having significant higher necromass than the treatment (Fig. 16).

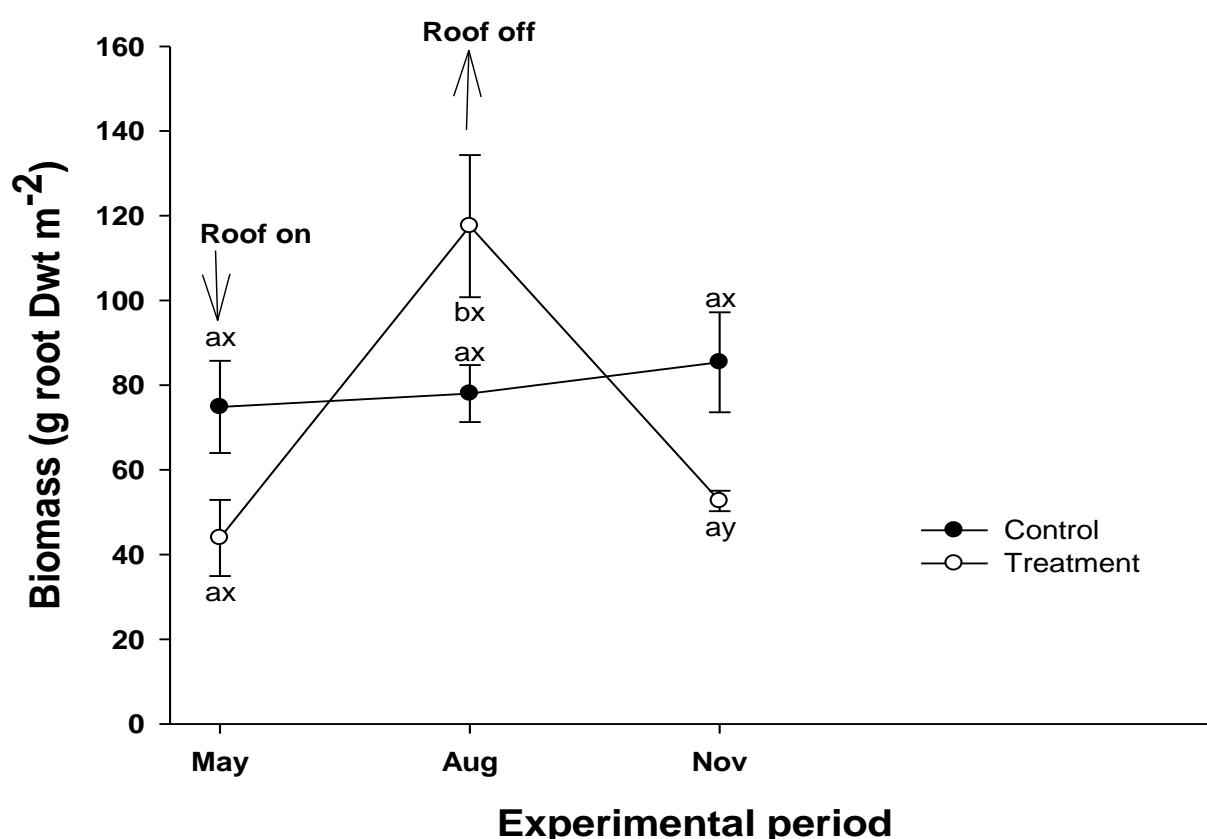


Figure 16: Total necromass in soil depth 0-30cm at Pangsho Gompa, in control and treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

The seasonal variation in necromass in control plots follows the trend: May < August < November. There was no significant difference in necromass seasonal variation in control plots. The seasonal variation in treatment plots follows the trend: May < August > November. August showed significant increase in necromass compared to May ($P= 0.003$), then it was followed by a significant decrease in November ($P=0.006$) in treatment plots (Fig. 16).

4.6 Pangsho Gompa, fine root biomass of dominant Species, 0-30cm

Quercus spp.:

Fine root of *Quercus spp.* in Pangsho Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 17. There was no significant difference in the fine root biomass of *Quercus spp.*, between the control and treatment plots, across any of the sampling dates.

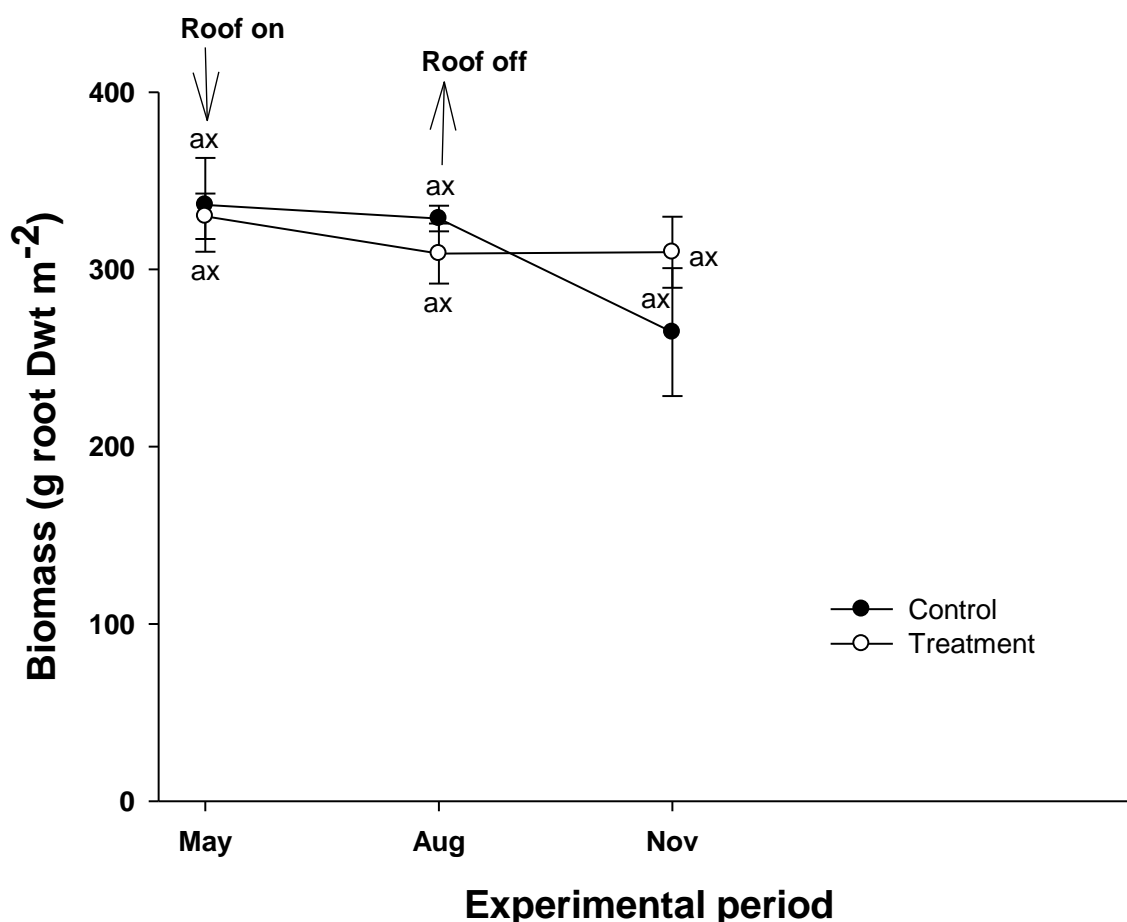


Figure 17: *Quercus spp.* fine root biomass in 0-30cm soil depth at Pangsho Gompa, in control and treatment plots across three time intervals. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

The seasonal variation in fine root biomass of *Quercus spp.* in both control and treatment follows the trend: May > August > November. The seasonal variation was statistically not significant (Fig. 17).

Rhododendron arboreum:

Fine root biomass of *Rhododendron arboreum* in Pangsho Gompa at three sampling dates viz.: May 2014, August 2014, November 2014 is shown in figure 18. There was no significant difference in the fine root biomass of *Rhododendron arboreum*, between the control and treatment plots, across any of the sampling dates.

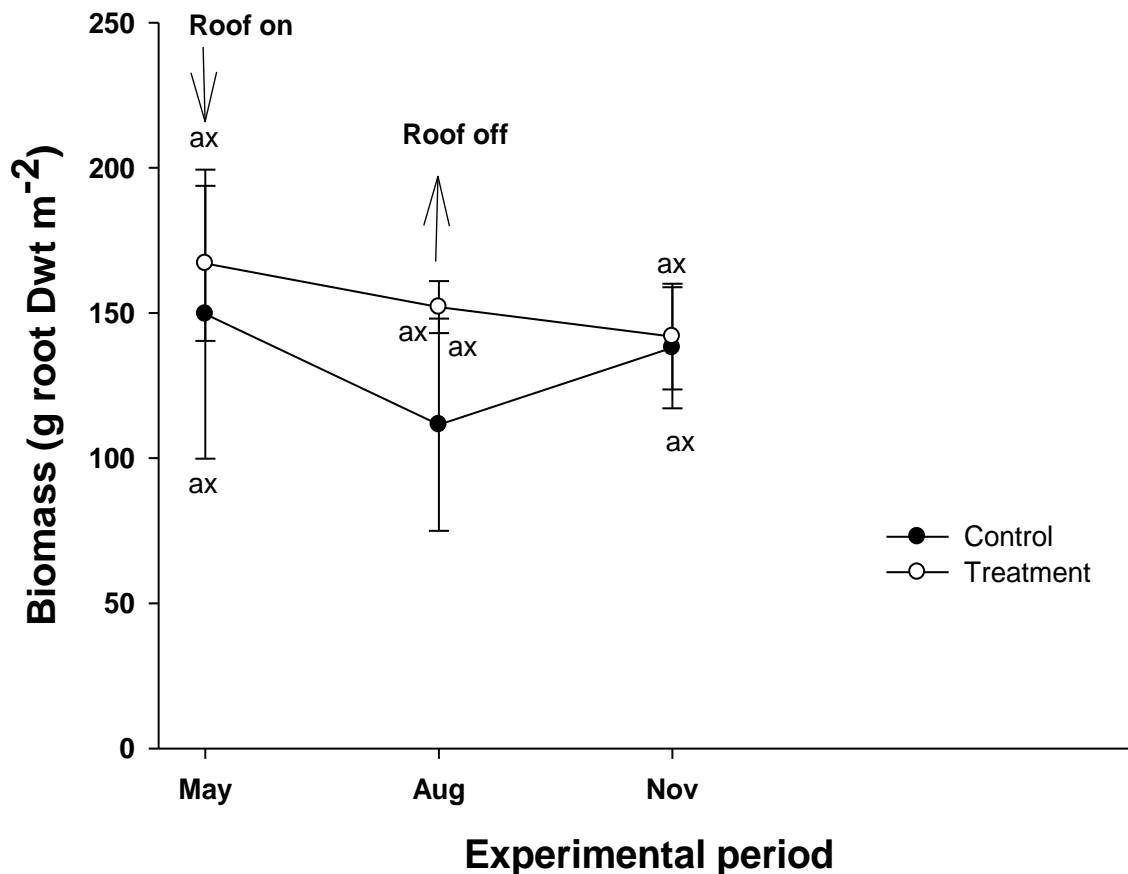


Figure 18: *Rhododendron arboreum* fine root biomass in 0-30cm soil depth at Pangsho Gompa, in control and treatment plots across three sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

The seasonal variation of *Rhododendron arboreum* fine root biomass in control follows the trend: May > August < November. And the trend for the treatment follows: May > August > November. However, the seasonal variation was statistically not significant (Fig. 18).

4.7 Pangsho Gompa, Vertical distribution of root biomass

Fine roots:

In case of fine root biomass, similar trend was noticed in all of the three sampling intervals. There was high significant effect of soil depth on fine root biomass at Pangsho Gompa ($P < 0.001$), the fine root biomass decreased with the increasing depth. The upper layer of 10cm had the largest portion of fine roots and least found in the deeper layer of 30cm soil depth. And there was no significant effect of the treatments on the fine root biomass (Fig. 19).

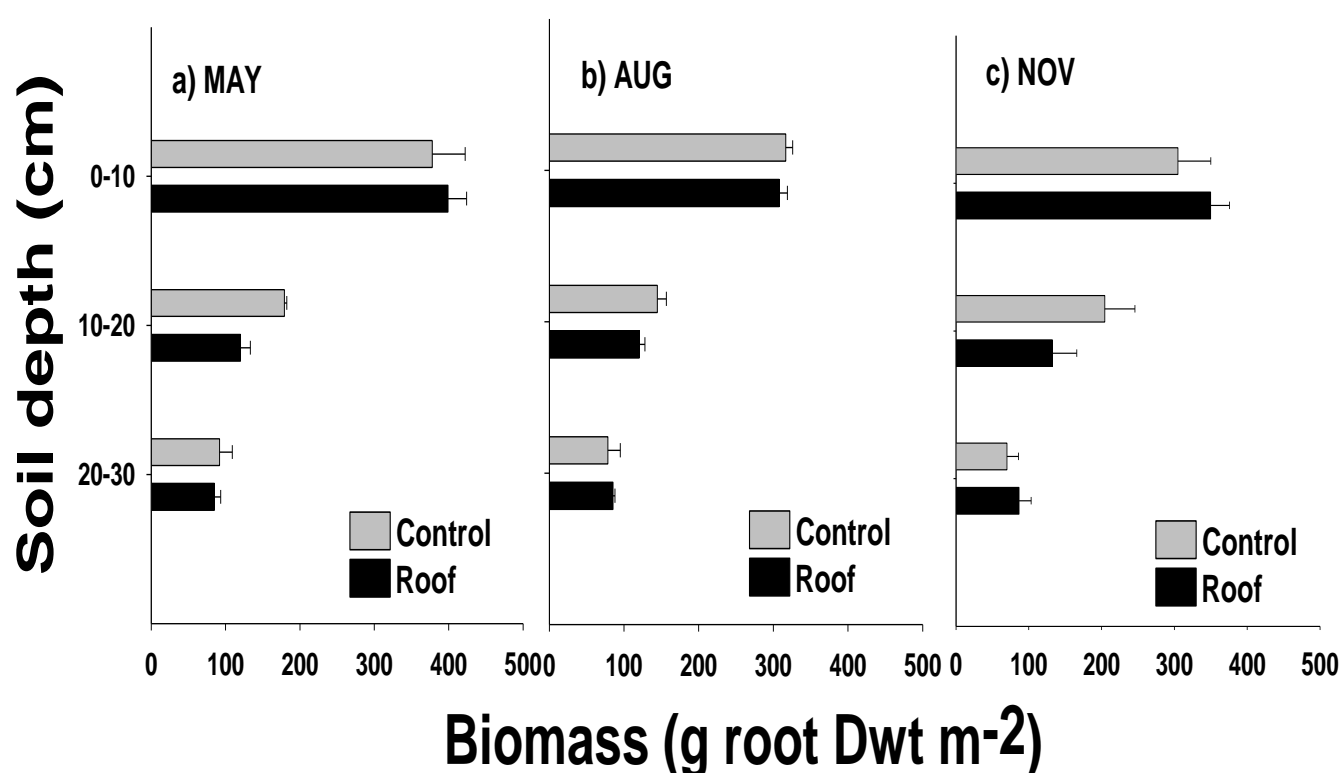


Figure 19: Depth wise distribution of fine root biomass at Pangsho Gompa in control and roof plots across three time sampling dates. Data points between sampling date (abc) or between treatments (xy) not followed by the same indices are significantly different ($P \leq 0.05$).

Necromass:

In May, no significant effect of either soil depth or treatments, on the necromass at Pangsho Gompa was observed (Fig. 20).

In August, there was a significant effect of both the soil depth and treatments on the necromass biomass. The necromass at the treatment plot was significantly higher than the control plot ($P=0.020$). And the necromass decreased with increasing depth, with P value of 0.045 (Fig. 20b).

In November, only the treatment had a significant effect on the necromass ($P= 0.028$), here the control plots had higher necromass than the roof plots (Fig. 20c).

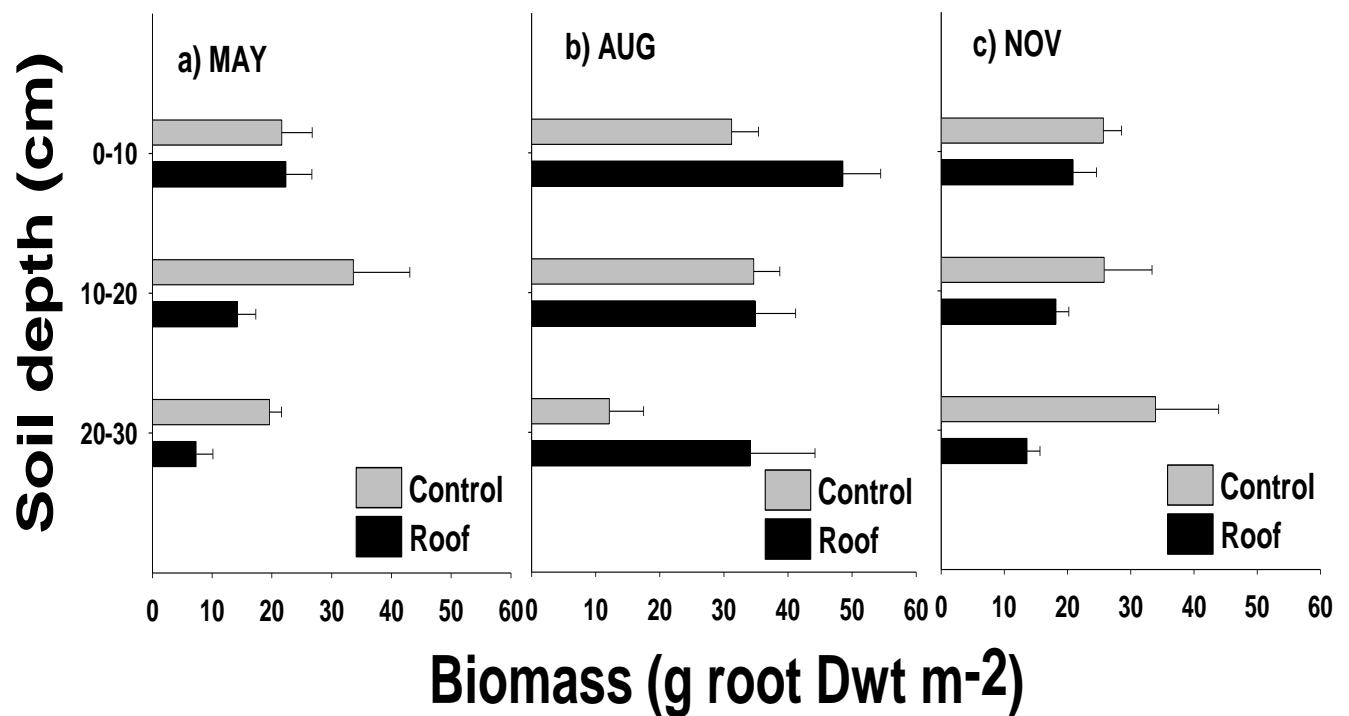


Figure 20: Depth wise distribution of necromass at Pangsho Gompa, in control and roof plots across three sampling dates.

4.8 Pangsho Gompa Species, Vertical distribution of fine root biomass

Quercus spp.:

In case of *Quercus spp.* fine root biomass, similar trend was shown in all of the three sampling dates. There was highly significant effect of soil depth on fine root biomass at Pangsho Gompa ($P < 0.001$). The fine roots decreased with the increasing depth, with major portion of the fine roots found at upper 10cm, and least amount in the 30cm soil depth. And there was no significant effect of the treatments on the *Quercus Spp.* fine root biomass (Fig. 21).

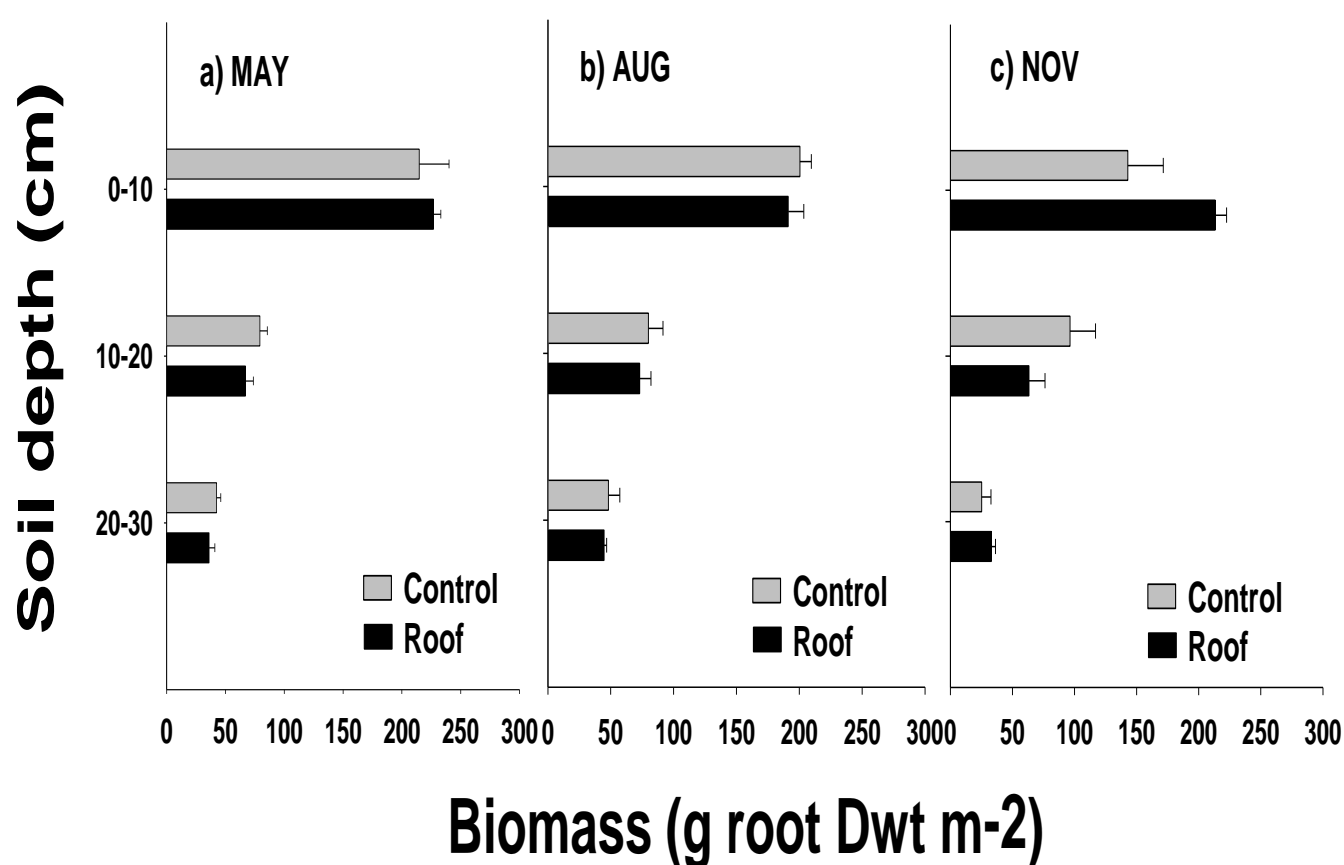


Figure 21: Depth wise distribution of fine root biomass of *Quercusspp.* at Pangsho Gompa, in control and roof plots across three sampling dates.

Rhododendron arboreum:

In case of *Rhododendron arboreum*, for all of the three sampling dates, there was a significant difference in the root biomass due to soil depth. The *Rhododendron arboreum* fine root biomass decreased with increasing depth, with major portion of the fine roots found at upper 10cm and least amount in the 30cm soil depth. And no significant difference was shown in the root biomass between the control and roof plots (Fig. 22).

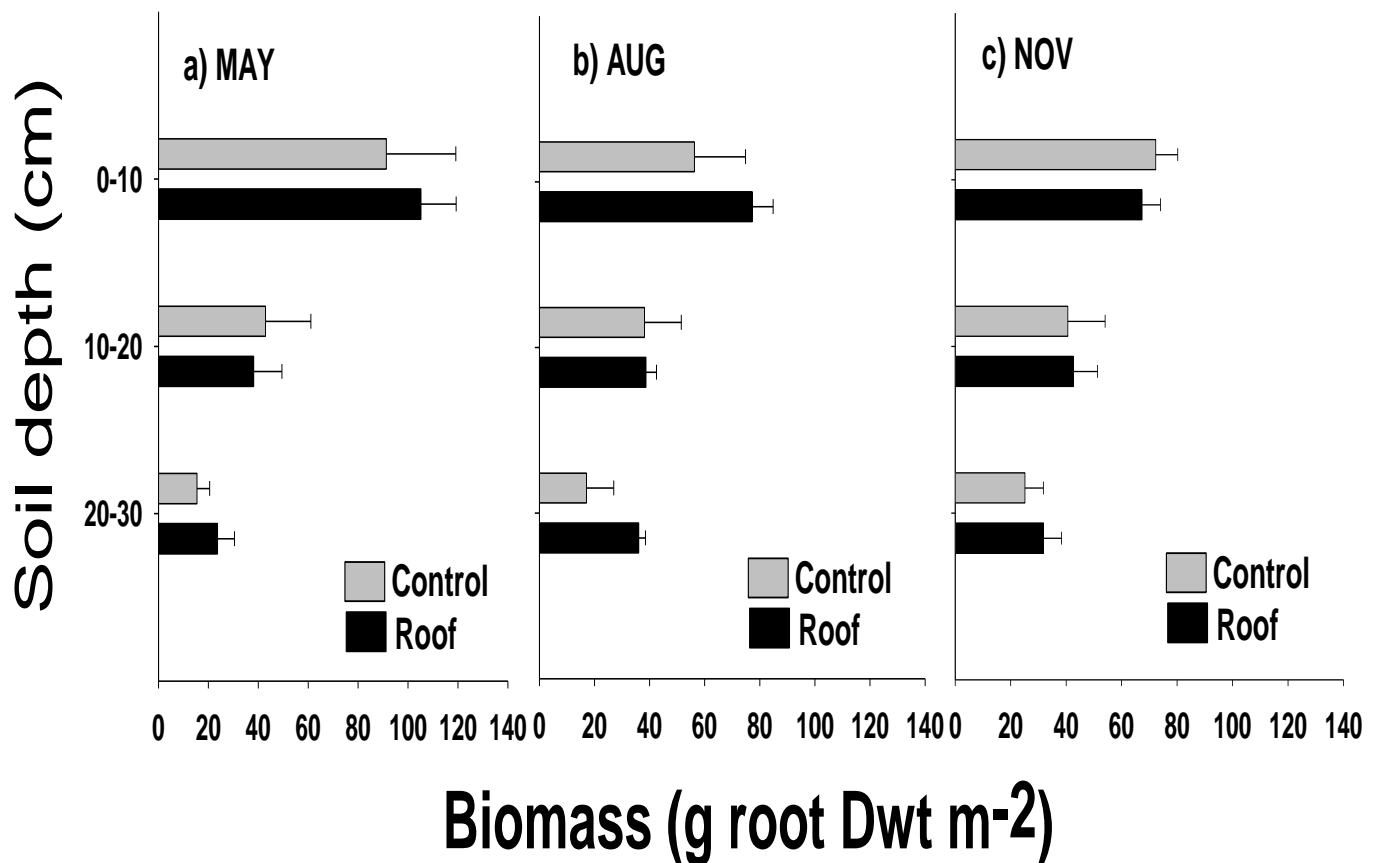


Figure 22: Depth wise distribution of *Rhododendron arboreum* fine root biomass at Pangsho Gompa, in control and roof plots across three sampling dates.

4.9 Total fine root biomass composition

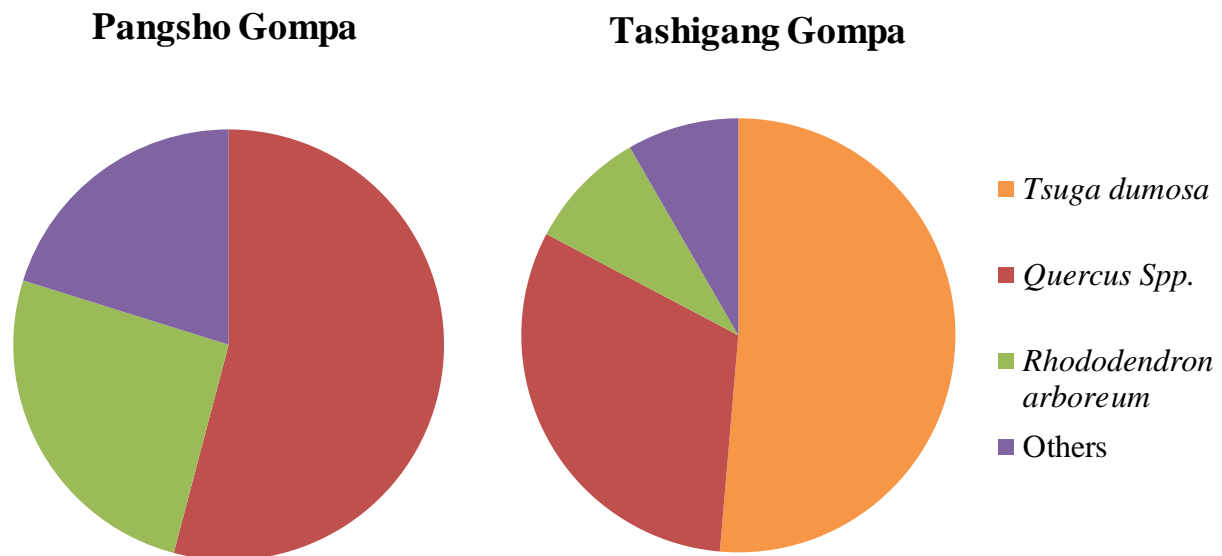


Figure 23: Relative proportion of total fine root biomass composition for dominant tree species at Pangsho Gompa and Tashigang Gompa research site.

In Tashigang Gompa, the total amount of fine root mass was found to be 416 g m^{-2} . The major portion of fine root biomass was of *Tsuga dumosa* with 51% (214 g m^{-2}), the next dominant fine root biomass was of *Quercus spp.* with 32% (130.6 g m^{-2}), then it was followed by *Rhododendron arboreum* with 9% (37.2 g m^{-2}). The fine root biomass of other species occupied 8% of the share (34.7 g m^{-2}).

In Pangsho Gompa, the total fine root biomass was found to be 615.7 g m^{-2} . The major portion of the fine root biomass belonged to *Quercus spp.* with 54% (333.1 g m^{-2}), it was followed by *Rhododendron arboreum* with 26% (158.3 g m^{-2}). The fine root biomass of other species occupied 20% of the share (124.2 g m^{-2}).

5 Discussion

5.1 Overall Fine root biomass findings

We stated that there will be effects of water stress on fine root biomass in a manipulated drought experiment along altitudinal gradient of Himalayan Bhutan. We hypothesised that the fine root biomass will significantly decrease in the drought experimental plots compared to control plots, due to decreased soil moisture. But we found that our research result do not support this hypothesis presented in the Introduction. Our results showed no significant difference in the fine root biomass, between the control and drought treatment plots. Secondly, there was no significant recovery of root biomass after the removal of roofs. Further, there was no significant difference in the fine root biomass, between the three sampling dates in both of the Tashigang Gumpa and Pangsho Gumpa sites (Fig. 7 and 14), indicating no seasonal variation of fine root biomass. Therefore we conclude that, there is no effect of drought on the fine root biomass, of the two forest types in Bhutan, for that experimental duration.

We expected the fine root biomass to significantly decrease in the drought treatment plots, because during the drought period, the root and leaves have less resources and nutrients to use, thereby leading them to have lesser metabolic activities. But certain studies suggest that drought may have negligible effects on fine root production over the longer term for trees growing in moist climates on sites with adequate moisture retention capacity (Joslin et al., 2000). Therefore, the reason for no significant decrease in the drought treatment plots could be attributed to the water holding capacity of the soil and tree species, as they can use these resources during moisture stress. However, we could see a general decreasing trend of the fine root biomass in the two research sites of Tashigang Gumpa and Pangsho Gumpa, for both the control and drought treatment plots. This indicates that, there is a less production of fine root biomass during that time of the season; July, August and September. This shows that, generally the root biomass decreases during the summer season despite the moisture availability (monsoon season). Similarly, such patterns of root biomass change have been identified for many ecosystems, for example, temperate deciduous trees are found to generally have a peak in root biomass prior to shoot growth in the spring, with maximum root growth often occurring in June or July and decreasing by August or September (Joslin et al., 2000). The reason could be that, the growing season of the species must be in spring, and after the spring season, the species generally decline in growth, hence leading to less root

biomass production generally. But there are lots of factors that could come to inter-play for such a result; the different species composition, shoot and root interaction, seasonal growth and different weather regimes.

A research done by Olesinski et al. (2011), investigated the effects of soil moisture on annual and seasonal fine root production and mortality over 4 years in a mature balsam fir stand (*Abies balsamea*) using a minirhizotron and soil coring. They found that the monthly fine root production was often reduced due to low soil water content, imposed by drought. They observed that, irrigation resulted in a higher fine root biomass production, and droughting decreased year-end fine root biomass in the treatment year. In the recovery year, the fine root biomass returned to the level of pre-treatment years. These findings, where the fine root production is reduced while the moisture stress is occurring, corroborates the findings of Blanck et al. (1995) in Norway spruce, and of Joslin and Wolfe (1998) and Joslin et al. (2000) in northern hardwood forest.

Contrast to the findings mentioned above, Pallardy and Rhoads (1993), reported that, exposure to drought had no effect on the subsequent root growth of seedlings of four different tree species. Similarly Bredemeier et al. (1998) observed no effect of prior artificial exposure to drought on subsequent Norway spruce (*Picea abies*) stand root production. Another research found that, the percentage of net primary productivity (NPP) allocated belowground over a 2 year period was significantly higher in non-irrigated Douglas fir (*Pseudotsuga menziesii*) stands than in irrigated stands (Gower et al., 1992). Further support comes from long-term irrigation studies done on coniferous forests as part of the EXMAN (Experimental manipulation of forest ecosystems) project in Europe; they found that, in three of these studies, irrigation treatments over 3 to 4 years diminished net root production relative to non-irrigated treatments (de Visser et al., 1994).

McCormack et al. (2014) found that, for many species, the pattern, timing and the total amount of root production varied substantially between years. However, basic patterns and variability in the rates and timing of fine root production and turnover are generally lacking in woody plants species. Therefore, the identification of these inter-annual patterns in root production is important and future efforts should focus on connecting these patterns with broader climatic variation (McCormack et al., 2014)

5.2 Spatial Variation of fine root biomass

Along altitudinal variations, there are changes of different environmental factors such as radiation, temperature and nutrient availability in soil, therefore the fine roots response to these changes also differs (Graefe et al., 2008). The lower temperatures and lower radiation inputs may reduce the decomposition rates and nutrient (Rosado et al., 2011). At higher altitudes, lower temperatures may promote the reduction in microbial and mycorrhizal fungi activities and their nutrient supply functions, leading to reductions in nutrient uptake (Leuschner et al., 2007). The differences in species composition between sites also play a major role in fine root production (Soethe et al., 2006).

In a global budget estimate for fine root biomass, Jackson et al. (1997), in their paper mentions that, in all of the biome, the live fine root biomass ranges from 130 g m^{-2} to 950 g m^{-2} , further in temperate coniferous forest the live root biomass is mentioned to be 500 g m^{-2} (Jackson et al., 1997). Our findings of fine root biomass also shows similar trend. The total fine root biomass of broad leaved forest at Pangsho Gompa site, which lies at an altitude of 2352m, was found to be 627.4 g m^{-2} (Fig. 14), and the total fine root biomass of cool conifer forest at Tashigang Gompa site, which lies at an altitude of 3290m, was found to be 466.2 g m^{-2} (Fig. 7). The fine root biomass at Pangsho Gompa was found to be 161.2 g m^{-2} higher than the Tashigang Gompa site. The difference may be attributed to the Pangsho Gompa being at lower altitude than the Tashigang Gompa. As stated above, the higher altitude makes the decomposition rates and the nutrient availability lower, decreasing the fine root production. Conversely, a research done in montane tropical forest in Brazil suggests that there will be higher fine root production with increase in altitude, in order to compensate the adverse environmental effects (Rosado et al., 2011). However a research done in tropical forests in Ecuador did not observe any increases in RLD along an altitudinal gradient from 1900m to 3000 m (Soethe et al., 2006).

Coarse roots are thought to live for significantly longer periods and their main role is related to resource transportation and tree stability. Their biomass will eventually contribute to soil biogeochemical cycles, but this usually happens after long time, maybe decades even, and their overall contribution to carbon and nutrient cycling is significantly smaller (Vogt et al., 1998). The coarse roots are also relatively higher in the Pangsho Gompa sites with 648.0 g m^{-2} than on Tashigang Gompa sites with 479.6 g m^{-2} . The same could be said for the necromass also, the Pangsho Gompa sites have higher necromass of 74.9 g m^{-2} and the Tashigang

Gompa sites have 46.3 g m^{-2} , indicating a higher decomposition rate at Pangsho Gompa than at Tashigang Gompa.

5.3 Depth wise distribution of fine root biomass

The evaluation of vertical fine root distribution (VRD) in the soil profile indicates the plant's resource use capacity in soil, the efficiency to avoid nutrient losses by leaching (Soethe et al., 2006), and the fine root's contribution to biogeochemical cycles (Jackson et al., 1996). Length densities of fine roots are an estimate for the spatial exploration of the soil by plant roots, and therefore the ability of plants to access nutrients at different soil depths (Bouillet et al., 2002). Further the vertical distribution is related to soil and plant characteristics including nutrient availability (Leuschner et al., 2007), soil pH (Godbold et al., 2003), access to water (Bouillet et al., 2002), waterlogging (Santiago, 2000), bulk density (de Oliveira Carvalheiro and Nepstad, 1996), stand age (Bouillet et al., 2002), species composition (Davis et al., 2004), and the type of mycorrhizal symbiosis (Moreno-Chacón and Lusk, 2004). It should be also noted, that the spatial availability of nutrients is also dependent on soil characteristics regulating nutrient transport to the root surface by diffusion, and on root characteristics such as root hair formation (Arvidsson, 1999).

In Pangsho Gompa site, the distribution of the fine root biomass was highest in the upper 0-10cm layer comprising of 62% and least in the 20-30cm layers with 14% of total fine root biomass (Fig. 19). The similar trend is followed by the Tashigang Gompa site, with highest found in the upper layer with 45.5%, and least in the 20-30cm layer with 26.2% of the total fine root biomass (Fig. 11). The differences are highly significant. The results shows that the Pangsho Gompa forest has a shallower rooting profile than the Tashigang Gompa forest, with 62% of the fine root occurring at the top 10cm. This overall result shows that, the maximum of the nutrients and nitrogen are located at the upper layers and least in the lower layers at both the altitudinal gradient. This high density of fine roots found in the top layers of the soil is important for the conservation of nutrients in the forests. And generally, the fine root biomass decreases with increased soil depth in many forest types (Borken et al., 2007, Børja et al., 2008). Similarly, the higher investment in fine roots at montane forests, especially in shallow soil layers to 0-5 cm depth, is probably a response to maximize the nutrient uptake under the lower decomposition rates (Rosado et al., 2011). And the higher content of organic matter at the montane site indicate a higher availability of more organic forms of nitrogen, which could be related to the higher investment in fine roots at 0-5 cm (Näsholm et al., 2009). There was no evidence of significant variation in root biomass due to drought treatment.

5.4 Dominant Species

Single species can substantially alter belowground processes in ecosystems through differential root production and mortality. However, our current information and understanding on species differences in fine-root demography is virtually absent for natural communities (Espeleta et al., 2009). The targeted research studies of belowground differences across species and resource gradients could advance our understanding of dynamic responses of terrestrial ecosystems to change, including shifts in species composition of natural communities (Espeleta et al., 2009).

5.4.1 *Tsuga dumosa*:

The results of the research shows that the fine root biomass of the *Tsuga dumosa* species at Tashigang Gompa site, has a decreasing trend in both the control and treatment plots, however there is no significant seasonal variation and also no significant difference in between the control and treatment plots (Fig. 10). There is no research done in the field of *Tsuga dumosa* fine roots, so no comparison could be made with other research. However our findings suggests that the fine roots production is maximum during the spring season, with 233.8 g m^{-2} during May and starts decreasing in the summer season, with the figure showing down to 193 g m^{-2} in the month of August. This means that the fine root growth of *Tsuga dumosa* is seasonal; it has highest fine root production in its flowering season, which is in the spring season of April to May, then afterwards its fine root production decreases.

In the depth wise distribution of *Tsuga dumosa* species at Tashigang Gompa, the upper layer of 0-10 cm contained a significant portion of 45% of the total fine root biomass compared to other depths (Fig. 13). However the result shows that, about 25% of the total fine root biomass is found at 20-30cm layer indicating a deeper root penetration for the absorption of water and nutrients. Several studies shows that, when trees experience moisture stress in one part of the rooting zone, the root growth increases in areas where water is still available, usually at greater depth (Hendrick and Pregitzer, 1996).

5.4.2 *Quercus* Species:

In the Oak dominated Pangsho Gompa site, two *Quercus* species were found, one is *Quercus lanata*, which is evergreen, and the other is *Quercus griffithii*, which is deciduous. They were found in equal proportions (Fig. 3). The result shows that, the fine roots biomass of the *Quercus Spp.* in the Pangsho Gompa site follows a decreasing trend from May till August, in

both the control and treatment plots. However, there was no significant seasonal variation, and also no significant difference in between the control and treatment plots (Fig. 17). Our findings suggests that the fine roots production is maximum during the spring season, with 336.4 g m^{-2} during May and slightly decreases in the summer season, with the figure showing 328.7 g m^{-2} in the month of August. This means that the fine root growth of *Quercus* species has not changed during the summer season.

The depth wise distribution of the *Quercus spp.* is highest on the 0-10cm soil depth with 66%, and lowest in the 20-30cm depth with only 11% (Fig. 21). This shows that the *Quercus spp.* at Pangsho Gompa forest has shallow rooting and penetrates less to the deeper layer compared to the *Tsuga dumosa* tree roots in the Tashigang Gompa forest. This also shows that there is higher nutrient and water availability in the top layers, than the deeper layers in the Pangsho Gompa forest.

5.4.3 *Rhododendron arboreum*:

The results of *rhododendron arboretum* fine root biomass at Pangsho Gompa site shows a decreasing trend in both the control and treatment plots from May till August, However there is no significant seasonal variation and also no significant difference in between the control and treatment plots. Our findings suggests that the fine roots production is maximum during the spring season, with 149.6 g m^{-2} during May and then starts decreasing in the summer season, with the figure showing down to 111.5 g m^{-2} in the August (Fig. 18). This means that the fine root growth of *Rhododendron arboreum* is seasonal; it has highest fine root production in its flowering season, which is in the spring season of April to May, then afterwards its fine root production decreases.

The depth wise distribution result of the *Rhododendron arboreum* shows, 62% of its fine root in the upper 10cm (Fig. 22). It follows the similar trend of *Quercus spp.* This shows that the *Rhododendron* species at Pangsho Gompa site has shallow rooting and penetrates less to the deeper layer. It means that there is higher availability of water and nutrients resources at the top layers of the Pangsho Gompa site.

5.5 Seasonal Variation of fine root biomass

Joslin et al. (2000) had an interesting finding from the observation of root elongation over 5 years. They found that a forest ecosystem has apparent resilience in ostensibly maintaining a relative constant fine root biomass over long-term. This resilience was most evident in the dry treatment. And it is dependent on the ability of the stand to respond with spurts of root

growth during period of high moisture and moderate temperature (Joslin et al., 2000). In our research, our result shows that, the seasonal variation of the fine root biomass shows no significant difference in the both the Tashigang Gomba and Pangsho Gomba sites. Further, there was no significant decrease in fine root biomass of the drought treatment plots compared to control plots across the three sampling dates, in both the research sites of Pangsho Gomba and Tashigang Gomba (Fig. 7 and 14). However, there was a slight decreasing trend in both the control and treatment plots, indicating the fact that there is a seasonal effect on the root biomass, causing them to decrease in production from May till November in the case of Tashigang Gomba, and May till August in case of Pangsho Gomba. But the exception is that, the fine root biomass shows an increase in the recovery period in the treatment plots at Tashigang Gomba. Such similar results of rise in fine root biomass during recovery periods are also shown by the *Tsugadumosa* at Tashigang Gomba, and *Quercus Spp.* and *Rhododendron arboreum* at Pangsho Gomba sites (Fig. 10, 14, 17 and 18). This suggests that, after a considerable stress of drought, the fine root production increases. This fine root production after a drought must be considered when evaluating tree growth. Joslin and Wolfe (1998) and Joslin et al. (2000), in their research, found enhanced fine root production after experimental droughts ended. They used the principle of a functional balance (Thornley, 1976), between foliage and fine roots to explain this response to moisture stress, they suggests that trees respond to modest, short-term moisture stress by enhancing fine root production later in the growing season in order to maintain the pre-existing balance rather than finding a new balance (Joslin et al., 2000).

Further when we look at the necromass biomass (Fig. 9 and 16), our result shows that there was a considerable rise in the necromass during the August interval (summer), when the temperature is at its peak in Bhutan. Gill and Jackson (2000), noted that one might expect a higher root mortality rates during periods of high soil temperatures since root respiration rates, nutrient mineralization rates, and pathogen and herbivore levels all increase with soil temperature. Thereafter in the month of November, in the recovery period, there was drastic decrease in the necromass. This low mortality rates in the recovery period must have contributed to the recovery and an increase of fine root biomass in the November period (Fig. 10, 14, 17 and 18).

5.6 Limitations of roof experiment

Despite the importance of fine roots, the difficulties associated with sampling and evaluation of different factors affecting fine roots parameters like species composition, soil moisture, nutrient and water availability, temperature and soil texture results in a lack of information for different environments (Soethe et al., 2006, Graefe et al., 2008). Warming and changes in irradiance may be part of the predicted climate change in certain scenarios, and one could say that such roof effects might help to simulate future climate more realistically. Nevertheless, all the environmental factors are not exactly controlled in roof experiments and may not mirror regional projections. The roof experiment may themselves affect ecosystems and therefore confound the results of drought experiments. Both the actual drought and roof experiments might differ in various aspects, for example, different plant communities, different temperature regimes and different disturbance regimes, and this therefore would further confound the results on interactions (Vogel et al., 2013).

The limitation associated with the roof experiments are, the roof experiments do not give much stress on the compounding effects of the radiation inside the roof, the irradiance would come inside the roof, but would be difficult for it to leave, because of the plastic roof above. This would lead to an increase of temperature, but again, the roof might act as a shelter from the heat of the sun, leading to lowering of temperature.

We must be cautious about drawing inferences about the role of drought in determining fine root productivity and possible tree growth responses to climate change from this drought experiment, because (i) we imposed drought during only one growing season, (ii) the drought was for a much shorter duration than would be expected to occur naturally, (iii) we measured recovery for only two months, and (iv) our sampling was limited by the number of established plots for this study.

6 Conclusion and recommendations

From our findings of the manipulated drought experiment, we can conclude that there is no effect of water stress on the fine root biomass of two different altitudinal gradient forest of Bhutan, namely Cool conifer forest at 3290m and mixed broad leaved forest at 2352m. Our result showed no significant difference between the control and drought treatment plots in any of the three sampling intervals. Moreover there is no evidence of significant difference in the seasonal variation of the fine root biomass.

The following are the list of recommendations for the future of the research to be carried out:

- i) Extension of number of plots: Presently we have two replicate for drought treatment and two replicates for control, in future one could increase the number replicate plots. We could increase to minimum of four replicates for each of the treatments. This is in order to have a good statistical analysis of the collected data and to minimize standard error.
- ii) Inclusion of more species: In Bhutan there is no research done on the fine root dynamics of any species. We could include other species found in the research sites apart from the only dominant species. There are other important species like Spruce (*Picea spinulosa*) and Fir (*Abies densa*) found at the sites. This could lead us to better understand the root characteristics of the species and their response to drought. And also studying more number of species would give us a conclusive and comprehensive understanding of the research.
- iii) Include other altitudinal locations: Presently we have two altitudes of 3290m at Tashigang Gompa and 2352m at Pangsho Gompa. The forest in Bhutan changes drastically due to even slight change in altitude; hence we have different forest types ranging from sub-tropical in the south to alpine in the north. Therefore in-order to have a conclusive and wider picture of the effect of monsoon drought in Bhutan, we need to add more altitudinal gradients or more forest types into the research.
- iv) Long term experiment: We need to do this study for longer duration in-order to have a consistent and reliable result. The long term research would enable to evaluate the findings over many seasons; hence we would be able to comprehensively observe the additive or quantified effect of the season on the fine root biomass over longer duration.

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- v) Include other parameters of root research: We should include other parameters and methods in evaluating the effect of drought on the fine root dynamics, this would give us a comprehensive picture and better understanding of the root dynamics. Other findings would involve a) Root turn-over b) Root decomposition c) Morphology of the roots.

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8 Acronyms

ANOVA- Analysis of Variance

ANPP- Aboveground Net Primary Productivity

EXMAN- Experimental manipulation of forest ecosystems

C - Carbon

C1 - Control Plot 1

C2 - Control Plot 2

ENSO- The El Niño–Southern Oscillation

Geog – Administrative centre for few villages

Gompa – Small Monastery.

IPCC- Intergovernmental Panel on Climate Change

LUPP- The Land Use Planning Project

MADA- Monsoon Asia Drought Atlas

NEE- Net Ecosystem Exchange

PG – Pangsho Gompa

RLD- Root Length Diameter

RNR-RDC- Renewable Natural Resources Research Development Centre

SPSS- Statistical Package for Social Science

TG – Tashigang Gompa

TFE- Through Fall Exclusion Experiment

T1 – Treatment Plot 1

T2 – Treatment Plot 2