UNIVERSIDAD DE CHILE
FACULTAD DE CIENCIAS AGRONÓMICAS
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VALIDATION OF NON-DESTRUCTIVE TECHNIQUES TO ESTIMATE THE NITROGEN RETENTION IN THE VEGETAL COMPONENTS OF BUFFER STRIPS

Tesis para optar al Título Profesional de Ingeniero Agrónomo y al Grado de Magíster en Manejo de Suelos y Aguas

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VALIDACIÓN DE TÉCNICAS NO DESTRUCTIVAS PARA ESTIMAR LA RETENCIÓN DE NITRÓGENO EN LOS COMPONENTES VEGETALES DE UN BIOFILTRO

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CHAPTER I. NON-DESTRUCTIVE TECHNIQUES TO ESTIMATE THE NITROGEN RETENTION IN THE VEGETAL COMPONENTS OF BUFFER STRIPS. A REVIEW

Nitrogen global situation

Nitrogen (N) is an essential nutrient for terrestrial and marine life, influencing many aspects of natural ecosystems and intervened by man (Vitousek e*t al.*, 1997). It is the nutrient that most often limits the primary productivity of ecosystems. Most N in the atmosphere is present as N_2, while other forms of N are found in small amounts. However, most organisms can only assimilate forms of reactive N (Nr), including oxidized and reduced inorganic and organic forms (Duce *et al.*, 2008).

Human activities during the last century has significantly increased the N forms available in the environment, in consequence generated a significant alteration of the global N cycle (Galloway and Cowling, 2002), which has increased to more than double the N involved in the environment (Collins *et al.*, 2010). Indeed, it is estimated that human derived N surpassed all natural processes combined sometime around the 1980s, a trend that is projected to increase Nr another 70% by 2050 (Galloway *et al.*, 2004, Vitousek *et al.*, 1997). While most of this fixed N initially enters the terrestrial biosphere, a portion is transferred from terrestrial to aquatic systems, resulting in increased N in streams and rivers that transport N to coastal ecosystems (Seitzinger *et al.*, 2002). According to some studies the contamination of waterbodies by N evolved from a local to a regional scale (Vitousek and Howarth, 1991; Heathwaite *et al.*, 1993; Webber *et al.*, 2010), nevertheless, recent research has demonstrated that the problem has reached a continental scale affecting large areas of the oceans and even that is a global problem (Doney, 2010). As a result human activities have increased total dissolved inorganic N loading above the natural fluxes by more than a factor of three, changes that are recognizable on time scales as short as two decades (Smith *et al.*, 2003). Furthermore, it has been seen that Nr input to oceans is rapidly approaching to the N_2 fixation estimates for global ocean and is predicted to increase even more (Duce *et al.*, 2008). The global trend is the increase of water pollution by N, which would be more intense according to the population density of each zone, largely associated with the change in land use, which usually means in an alteration of the nutrient concentrations, particularly N and phosphorus (P) in the waterbodies (Oyarzún *et al.*, 1997). It has been found that deforestation leads to increase nitrate (NO_3^-) concentrations in the water because of the intensification of the soil erosion, increased surface runoff, the decomposition of plant matter and reduced absorption of nutrients. For instance, Seitzinger *et al*. (2002) found that total dissolved inorganic N export into the ocean from South America is 1.5 teragram (Tg) N yr^{-1}, while Eastern Asia exported approximately 5.3 Tg N yr^{-1}, a similar amount exported by Southern Asia, more densely populated areas.

Human sources of N

In water pollution act various human activities as sources of N to the environment, such as industrial activity, discharges of waste water, production and use of fertilizers, use of fossil fuels, application of organic manures and other organic waste. Among these activities, some act as point sources of pollution (PSP) and / or diffuse sources of pollution (DSP), which cause severe damage to coastal ecosystems and threaten drinking water supplies for population (Wu and Chen, 2013). The PSP mainly include municipal waste discharges (urban or residential areas) and wastewater discharges from industrial areas. The DSP originate from the movement of rainwater, thawing or irrigation water through the soil, which transported and deposited pollutants in rivers, lakes and coastal areas (Wu and Chen, 2013). Until some years, the global focus of pollution of waterbodies, was the PSP; however, currently exists a growing concern about the DSP, mainly from agricultural land. Moreover, human activity also is accelerating the release of N from storage sites such as soil organic matter (Vitousek *et al*., 1997).

Before the green revolution, the biological fixation was the largest source of N involved in the biogeochemical cycles. After the green revolution, the utilization of pesticides and fertilizers allowed to increase the agricultural productivity, but also impose a negative impact on surface water quality, biodiversity and biological control potential. According to Galloway (1998), between 1950 and 1990 the per capita use of N fertilizer increased about ten times, from about 1.3 to 15 kg N person^{-1}. Fertilizer in the forms of ammonium nitrate, urea, calcium nitrate, ammonium bicarbonate and several varieties of NPK fertilizers correspond to 80% of the chemical production of ammonium (NH_4^+), from the Haber-Bosch process (Erisman *et al*., 2007). As demands for food and energy continue to increase, both the amount of Nr and the magnitude of the consequences will also increase (Galloway *et al*., 2008).

Environmental effects of N pollution

Although the benefits of N added to cropping systems are clear, it also well-documented its environmental costs: increased coastal hypoxia, atmospheric nitrous oxide (N_2O), Nr gases in the troposphere, decrease in water quality, N deposition onto forests and other natural areas (Robertson and Vitousek, 2009). On an annual basis, only the 38% of agricultural N inputs in the United States enter the annual food and livestock feed supply, additionally estimate that approximately 13% is released into the surface waters and groundwater by leaching (Suddick *et al*., 2012). Ribbe *et al*. (2008) conclude that 23-27% of the N fertilizer applied to the fields is leached to the surface water. In consequence, the agricultural activity is emerging as one of the mayor sources of non-point contamination by nitrogenous elements in water in the USA, the European Union and the world (Selman *et al*., 2008).

It is well known that the main factor of water pollution by fertilizer in the agricultural activity is the low efficiency in their use, when it has been reported that efficiency ranged from 20 to 30% under rainfed conditions and 30 to 40% under irrigated conditions (Mujeri *et al*., 2012). This means that most of the N fertilizer applied is loss by lixiviation, surface runoff or lost to the atmosphere through the denitrification, before being assimilated to biomass (Canfield *et al*., 2010). The main form of N in water is NO_3^-, which goes to water due of a temporally coincidence of the anion in the soil with a rain event or irrigation. The pollution of surface or ground water generates as much problems, not only to the environment also in human health. This pollution of aquatic ecosystems may result in three major environmental problems: water acidification, eutrophication and direct toxicity of inorganic nitrogenous compounds (Camargo and Alonso, 2006). It is important to note that the presence of NO_3^- in surface water generates eutrophication, which is an enrichment of N and P in water, that causes an increment in the growth of algae and higher forms of plant life, which causes a negative disturbance in the equilibrium of the organisms present in water (Andersen e*t al*., 2006). The NO_3^- present in groundwater, which are used for human consumption, is related to severe health problems as gastric and colon cancer (Ryczel, 2006) and can induced methemoglobinemia, particularly in young infants, by blocking the oxygen-carrying capacity of hemoglobin (Camargo and Alonso, 2006). For all of the previous reasons is imperative to establish an efficient management of N fertilizer, to achieve an optimum productivity, preserving the environment quality as an important objective in modern agricultural systems (Jifon *et al*., 2005).

Nitrogen national situation

The situation in Chile is similar that in other parts of the world, especially in the central-southern region, which historically have focused the main productive activities, among them agriculture, forestry and livestock. In addition, their intensities have been increasing over time as population growth in the zone, this is why, it should be expected a significant increase in the levels of N and P in Chilean rivers (Pizarro *et al*., 2010). Furthermore between the Metropolitan Region and Los Lagos Region large amounts of fertilizer are applied, concentrating more than 90% of the national consumption of N fertilizer and using low technological levels of irrigation (Arumi *et al*., 2005). The historic fertilizer consumption in Chile had been shown significant increases. In 1980 N and P consumption was approximately 50 to 70 thousand tons respectively, while in 1988 the consumption increased to around 150 and 145 thousand tons respectively (Baherle y Landon, 1989). For its part in 2014 fertilizer imports totalled 1.062.000 tons, with about 573 and 317 thousand tons of N and P fertilizers, respectively (Espinoza and Valdés, 2015).

In Chile, few studies have been conducted for evaluate the impact of agricultural activities in NO_3^- levels in water bodies. A study in the Valparaíso and Metropolitana Regions of Central Chile found that high concentrations of NO_3^- and phosphate occur downstream of agricultural areas, as consequence of fertilizers (Jorquera *et al*., 2014). In a study in the drainage system of Rupanco Lake, Los Lagos Region, Oyarzún *et al.* (1997) evaluated the effect of land use on the concentrations and export of N and P from six micro watersheds. They found that the NO_3^- represented the highest values of all N forms in water, especially in the basins with agricultural activities. In the same Lake the exports of NO_3^- and total Nitrogen (TN) concentrations from 4 tributary sub watersheds increased from 14 N-NO_3 kg km^{-2} yr^{-1} and 33 TN kg km^{-2} y^{-1}; whereas in the most pristine sub watersheds, to 340 N-NO_3 kg km^{-2} yr^{-1} and 621 TN kg km^{-2} y^{-1} in the sub watersheds with the greatest surface of crop and pasture lands (Léon-Muñoz *et al*., 2013). Pizarro *et al.* (2010), considering data from 23 years of the main basins of the country, found that the basins of the rivers Biobío, Bueno, Imperial, Maule, Rapel and Valdivia show a clear increase in the concentrations of NO_3^-. In the basin of Pocochay, Valparaíso Region, Ribbe *et al.* (2008) found NO_3^- concentrations ranging from 1.3 to 10.1 mg L^{-1}, where the authors noticed that in the basin around 52 kg N ha^{-1} are drained compared with 167 kg N ha^{-1} applied as fertilizer on fields.

Other studies carried out by Golembesky (2004), Iriarte (2007) and Corradini *et al.* (2015) found that the measured NO_3^- values in water bodies adjacent to agricultural areas in the Mediterranean zone of Chile, where maize is the most common crop in the area, were over to the quality standard of the Chilean drinking water (>10 mg L^{-1} of $N\text{-}NO_3$). In addition, more than 40% of the water consumed in urban zones of Chile is groundwater, and in the case of rural areas, this percentage increases to 76% (Arumi *et al.*, 2005). In addition, Arumi *et al*. (2006) carried out a study in the zone of Parral, Maule Region, where found that 14% of the monitored wells had NO_3^- concentration values greater than those permitted by national regulations for drinking water. However, Arumi *et al.* (2005) considers that in general in the Chilean central valley not present high values of NO_3^- in groundwater.

Best management practices

With the purpose of avoiding the diffuse pollution of water, several techniques have been used in rural and urban areas. Because the movement of N dissolved in water from fields to water bodies occurs through the surface runoff and subsurface lateral flow, the strategies to use must seek to intercept and retain the N forms present in water before it reaches a nearby water bodies. In this sense in rural field the N fertilizer application to crops must be calculated an appropriate N dose considering the needs of N by the crop, the supply provided by the soil and the application efficiency of N, in order to minimize losses of N to the environment (Chaubey *et al.*, 1994). In addition, the use of slow delivery fertilizers, split-N applications, the use of non-chemical fertilizer and other practices such as an adequate and efficient irrigation system can help to diminish the N losses and further pollution of water. However, these practices are not entirely effective to retain the N that goes to the limits of the field by surface runoff and subsurface lateral flow, so it cannot prevent movement towards nearby surface waters. Therefore, it is important to note that independent of N management on field, losses of this element will always be generated as a natural process and will be intensified by mismanagement.

In addition, conventional practices such as dry and wet ponds, and more innovating alternatives such as green roofs, permeable pavement, bioretention, vegetated open channels, sand filters and wetlands are being developed at global level (Collins *et al.*, 2010). Other possible alternatives are the vegetative buffers strips (BS) that are promising for reducing erosion, agro-chemical runoff and leaching processes. The BS are permanent vegetation stripes that include trees, bushes and prairies usually close to water bodies (Mayer *et al.*, 2006). The BS are characterized by high density and diversity of species, which are located in a transition zone, specifically at the interface between terrestrial and aquatic ecosystems (Burt and Haycock, 1993). In these buffer zones, soil presents a continuous or periodic saturation condition, caused by groundwater or capillary rise (Lin *et al.*, 2002) determining anaerobic conditions in the zone.

The BS act as a biochemical and physical barrier among the potential sources of contamination and the adjacent open water system (van Beek *et al.*, 2007). The runoff flow through them and the N can be eliminates via: assimilation, absorption and denitrification (Collins *et al.*, 2010). Hence, the removal of N from surface flows is produced by sediment deposition that have N adsorbed to its surface and/or the exchange of N dissolved with the soil surface and organic residues (Mihara, 2006). In addition, BS furnish the transformation and immobilization of N dissolved through subsurface flows due to the action of soil microorganisms and the vegetation (Rassam *et al.*, 2006). A vegetated surface increases removal through uptake of pollutants in addition to impact velocity and infiltration processes (Larson and Safferman, 2012).

The most important factors that determine the effectiveness of the BS are the movement of water through or over them (hydrology), the width of BS and plant composition.

To remove the N in surface runoff is necessary that the velocity of surface flow decreases enough to allow sediment deposition and its stabilizing on the buffer zone in order to avoid channelled flowing fast (Dillaha *et al.*, 1989). One of the most important environmental parameters governing the effectiveness of the BS is the width (Rasmussen *et al*, 2011). The N retention of the surface runoff fluctuates between 10% to 80% (Shirley and Smith, 2005), where the buffer N retention capacity increases when the vegetative zone grows (Otto *et al*, 2008). For example, studies in the US (Mayer *et al*, 2006) noted that wider strips (> 50 m) consequently removed a significantly greater amount of N. The width of the BS is an important variable because it affects the time of dwell between the water that is transported to the water bodies and the vegetation of the BS. This period will increase, as the width also increases (Grismer *et al.*, 2006). However, the establishment of BS must be adequate to the particular situation of each zone where is going to be carried out. For example, in Italy because of the fragmentation of land ownership, the BS are lesser than the ones used in the USA. In this country the studies developed have evaluated narrow BS that goes from the range of 5 m to 8 m (Borin and Bigon, 2002; Borin *et al.,* 2005; Balestrini *et al.,* 2011), reporting a high effectiveness in NO_3^- removal. Similarly, a pilot experiment was carried out in Central Chile with promising results for reducing N losses (Tapia and Villavicencio, 2007). Syversen (2005) determined the existence of differences between widths of the BS concluding that a narrow strip (5-10 m) is effective to catch particles and nutrients. However, another study (Dunn *et al.*, 2011) found that both the slope of the field as the width of the BS did not affect its ability to reduce pollutants from agricultural soils.

The type of vegetation that is found in the BS is the primary importance in controlling the efficiency of NO_3^- absorption process within this terrestrial-aquatic interface (Burt and Haycock, 1993). For example, Wang *et al.* (2012) found that tree and pasture together, reduced NO_3^- concentration from the surface runoff, shallow and deep groundwater by rates of 9%, 15% and 14% respectively, indicating that vegetated BS has an efficient function to reduce N pollution. Another authors, Duchemin and Hoguen (2009) compared two BS with a different plant composition, where the first was composed exclusively of prairie and the second by poplars and prairie. However, these authors found that the introduction of the poplars did not generate a significant increase in the capacity of the BS to retain N, possibly because trees were young and do not explored all the volume of soil. Thus, in some studies it was established that BS with trees and prairie are significantly effective to decrease the concentrations of NO_3^- in water flows, mainly through the reduction in the volume of surface runoff (Wang *et al.*, 2012; Fortier *et al.*, 2010). Additionally, other studies have discussed that the presence of trees is crucial in BS, because they can increase potential denitrification in these areas as they are the primary source of carbon to the soil at deeper horizons (Cuffney, 1988; Burt and Haycock, 1993). Balestrini *et al*. (2011) found that water absorption by trees affects subsurface flow patterns and contributes to remove the NO_3^-. Thus, there are trees and bushes than can improve the denitrification capacity in the BS (Cuffney, 1988), meanwhile the prairie acts intercepting the runoff and absorbing the N.

On the one hand, Hefting *et al*. (2005) mention that the N uptake by the plant in the buffer zone is generally considered less important in the ability to remove N denitrification due to the temporary retention of this element in the BS, since the N returns to be available in the field once the plant dies. On the other hand, it must consider the indirect effect of the vegetation in the denitrification by promoting the process through the release of organic matter to the soil (Balestrini *et al.*, 2011). In addition, surface runoff is intercepted by prairie and also have dense roots systems in the topsoil to promote absorption of N. One of the species most used in BS is the fescue (*Festuca arundinacea* Schreb), whose ability to absorb N and his adaptation to a wide range of soil types have been successfully tested in numerous studies (Daniels and Gilliam, 1996; Schmitt *et al.*, 1999; Vidon and Hill, 2004; Dunn *et al.*, 2011).

Furthermore, the good handling of the vegetative stripe can produce other benefits such as mitigate the diversity loss providing refuge and food for invertebrates, small mammals and birds (Josefsson *et al.*, 2013), wood production, acting like CO_2 sinks and improve landscape beauty (Borin *et al.*, 2010). So the BS contribute to mitigate negative environmental impacts of farming through provisioning of multiple ecosystem services in structurally complex agricultural landscapes (Josefsson *et al.*, 2013) and the economic benefits, make this an interesting way to mitigate the N loss by converting the zone, from traditional agricultural to a BS (Fortier *et al.*, 2010). Considering the above, the use of native species is important for incorporated further benefits to the BS, also native species can be more adapted to specific environmental conditions and be more successful on the BS. Among the native species with potential to use like component of BS can found two native tree species with habitat in Central Chile, which can tolerate prolonged periods of water logging, such as: “chequén” (*Luma chequen* (Mol.) A. Gray) and “canelo” (*Drimys winteri* J.R. et G. Forster). *L. chequen* is considered a riparian vegetation and a common species in swamp distributed along the coast of north-central Chile from Coquimbo to Los Lagos Region (31-41ºS). The *L. chequen* grows in permanently or semi-permanently flooded soils (Bascuñan *et al.*, 2013). *D. winteri* develops in different types of soils, but preferably in low and humid areas. It is adapted well to the upper parts of the hills in the southern rainforest and is under strong sunlight near rivers (Rodriguez, 1998).

Over the past 50 years the main studies in BS focus in N uptake, but rarely consider the influence of plant physiological traits such as nutrient assimilation on the effectiveness of nutrient reduction (Wang *et al.*, 2012). In this way, the N retention by vegetal components must be considered an important way to reduce N in water bodies, because of this is important to estimate the N excess that is retained in them. However, improving vegetation BS performance require a better understanding of the interaction between N inputs entering to buffer areas and timing with seasonal changes in vegetal components N demand.

Total nitrogen determination

Usually the methods employed for determination of N status in vegetal tissues (amount of N retained) are accurate but at the same time are destructive and its quantification are done in laboratory which is time consuming and high costly and do not allow repetitive measurement of the same sample (Da Silva *et al.*, 2012). Actually, non-destructive methods are interesting options to estimates the N status in plants. Different non-destructive methods have been testing such as spectroradiometers, reflectometers, images from satellite sensors and digital cameras using these meters optical properties have been measured to estimate N in plants, such as crop canopy reflectance, leaf transmittance, chlorophyll (Chl) and polyphenol fluorescence (Muñoz-Huerta *et al.*, 2013). Hand-held chlorophyll meters based on the Chl status that furnishes and indirect evaluation of N content because a great amount of N is incorporated in Chl. Both, Chl and N status of leaves provide valuable information about the physiological condition of plants (Pal *et al.*, 2012). Moreover, hand-held chlorophyll meters have been successfully used to estimate N concentration in a wide range of vegetal species, where leaf Chl content can be closely correlated with leaf N content (Chang and Robison, 2003; Girma *et al.*, 2006; Pinkard *et al.*, 2006). Hence, it is necessary to establish a mathematical relationship between device measurements and concentration of Chl or N making that an indirect estimation of N status may be possible. With portable chlorophyll meter therefore further simplifies quantifying N deficiency, making real-time, in the field diagnosis possible and allowing the promptly correct swards N deficiencies through fertilization (Errecar *et al*., 2012).

Non-destructive optical techniques based on leaf absorbance and reflectance of light by leaves have been proven as alternative time-saving and simple techniques, one of this portable chlorophyll meter is CCM-200 of Opti-Sciences enterprises. This device is based on th calculation of chlorophyll content ratio from the measurements of absorbance at red (660 nm) and near-infrared (NIR) (940 nm) (Richardson *et al.*, 2002) and the sampling area is of 71 mm^2 bigger than other meters. Increased chlorophyll concentration increases the absorption of red radiation also plants transmit a high fraction of NIR radiation and this transmission is used as a reference wavelength (Parry *et al.*, 2014). The equipment generates a Chl Content Index (CCI) value that indicates the relative Chl content but not absolute Chl content per unit leaf area, or concentration per unit of leaf mass (Pal *et al.*, 2012). *In situ* optical meters are widely used to estimate leaf Chl concentration, but a non-uniform Chl distribution causes optical measurements to vary widely among species for the same chlorophyll concentration (Parry *et al.*, 2014). The mathematical relationships between CCI value and total Chl or N contents vary with species and growing conditions. Thus, an independent mathematical model should be established for each species for a particular zone. The reported mathematical model of optical/absolute chlorophyll concentration relationship has varied widely, sometimes even within the same species (Parry *et al.*, 2014).

The equipment CCM-200 have been proven as alternative to quantify Chl in a number of agricultural species as *Citrus* sp. (Jifon *et al*., 2005), *Rosa damascena* (Pal *et al*., 2012), *Vitis vinifera* (Callejas *et al.*, 2013; Cerovic *et al*., 2012), *Actinidia deliciosa, Zea mays* (Cerovic *et al*., 2012) and *Triticum aestivum* (Cerovic *et al.*, 2012; Lunagaria *et al*., 2015). Also it has been reported strong relationship ($r^2 > 0{,}5$) between CCI and nitrogen status in *Citrus* sp. (Jifon *et al.*, 2005), *Cynara cardunculus* (Rodrigo *et al.*, 2007) and *Rosa damascena* (Pal *et al.*, 2012). Additionally, compared to other chlorophyll meters, several authors (Cate and Perkins, 2003; Biber, 2007; Ghasemi *et al.*, 2011; de Carvalho *et al.*, 2008) have successfully calibrated this device in tree species.

In addition, leaf colour chart (LCC) has already shown promise for improving N use efficiency in wheat, maize and rice (Maiti *et al.*, 2004; Islam *et al.*, 2007; Singh *et al.*, 2011; Singh *et al.*, 2012). Furthermore, these site-specific N management strategies include an element of prediction of yield potential during crop growth season (Ali *et al.*, 2014). As CCM-200, LCC is an easy to use and inexpensive diagnostic tool and proved quick and reliable use (Yosef Tabar, 2013). The LCC readings are a measure of leaf greenness, which is regulated by N availability and may be closely related to N status of plant (Gomara, 2012). Moreover, to know the total N retention of a system, it is necessary to establish the total biomass of the vegetal component.

Because N status assessment of plant biomass requires destructive harvest, drying and weight, which are tedious and labour-intensive approaches and produce a significant disturbance of the study sites (Northup *et al.*, 2005), an alternative approach is to measure non-destructive attributes of plant (Height, canopy area, diameter of basal trunk, etc.) and relate these reads to biomass through allometric equations developed from destructive sampling of plants. For quantification of tree biomass a large quantity of allometric biomass equation has been developed during the last decades (Porté *et al.*, 2002; López *et al.*, 2003; Zhou *et al.*, 2007; Návar, 2009; Sampaio *et al.*, 2010). Although the allometric data available for native species, specifically for economicall secondary species such as *D. winteri* and *L. chequen* is limited.

Norhtup *et al.* (2005) noted that canopy area produced more precise estimates of above-ground biomass of different shrub species than stem basal diameter, however both have considerations, metrics based on stem basal diameter are less sensitive to short-term environmental fluctuations, but it is more slowly to measure. Other authors reported good results for height and diameter at breast height (Hossain *et al.*, 2015). Also Roccuzzo *et al*. (2012) found good correlation for the first order branches cross-section in *Citrus sinensis.* Therefore, it is important to develop allometric equations for each species and for specific environmental conditions.

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CHAPTER II: CALIBRATION OF NON-DESTRUCTIVE TECHNIQUES TO ESTIMATE THE NITROGEN RETENTION IN THE VEGETAL COMPONENTS OF BUFFER STRIPS

ABSTRACT

Nitrogen (N) losses from fields are one of the main sources of diffuse pollution of water bodies in the world. A number of methods for reduction diffuse pollution have been developed, where buffer strips (BS) are a promising method. The vegetal components of BS intercept and assimilate the N forms that are moving through the surface runoff and the sub superficial lateral water flow diminishing the N load to surface waters. However, there is lack of information about the N retention capacity of the vegetation components of a BS. The main aim of this study was to develop equations to estimate the dry matter (DM) biomass and total nitrogen (TN) content of different vegetal components in a BS. In this study, two Chilean native trees species (*Drymis winteri* J.R. Forst. & G. Forst. y *Luma chequen* (Molina) A. Gray) and a prairie (*Festuca arundinaceae* Schreb.) were evaluated. The CCM-200 and Munsell System Leaf Color (MSLC) were used to estimate the TN content in trees and prairie, respectively. For the DM of trees two allometric variables, basal trunk diameter (BD) and height (H) were evaluated. There were found significant relationships (p<0.05) between CCM-200 readings and measured TN content of leaves for *D. winteri* and *L. chequen* with R^2_{adj} of 0.56 and 0.54, respectively. There was also found a significant relationship (p<0.05) for MSLC and TN content of *F. arundinaceae* (R^2_{adj}: 0.51). However, the results suggest that these non-destructive methods can under or overestimate the TN content due to intrinsic characteristics of leaves. In the development of equations to estimate the DM, it was found that only the BD presented significance relationships (p<0.05) to predict the DM of leaves, stem and roots of *D. winteri* and *L. chequen*. For *D. winteri* the R^2_{adj} ranged between 0.81 and 0.85 and for *L. chequen* the R^2_{adj} were 0.15, 0.86 and 0.53 for leaves, stem and roots, respectively. The results suggest that non-destructive techniques are useful for the biomass and TN content estimations. However N predictions present limitations associated with leaf characteristics influenced by environmental factors and may be necessary the development of more specific calibration equations.

Keywords: Allometric equations, Chlorophyll Content Meter, Munsell System Leaf Color.

CALIBRACIÓN DE TÉCNICAS NO DESTRUCTIVAS PARA ESTIMAR LA RETENCIÓN DE NITRÓGENO EN LOS COMPONENTES VEGETALES DE UN BIOFILTRO

RESUMEN

Las zonas agrícolas son una de las principales fuentes de contaminación difusa de cuerpos de agua en el mundo, debido a las pérdidas de nitrógeno (N) que en ellas ocurren. Numerosos métodos para reducir la contaminación difusa se han desarrollado, donde el uso de biofiltros (BS) aparece como una técnica relevante. Los componentes vegetales del BS interceptan y asimilan formas nitrogenadas que se están moviendo a través de la escorrentía superficial y los flujos subsuperficiales laterales, disminuyendo las carga de N que llegan a cuerpos de agua superficiales. Sin embargo, existe poca información acerca la capacidad de retención de N de los componentes vegetales de BS. El objetivo principal de este estudio fue desarrollar ecuaciones para estimar la materia seca (DM) y el contenido de nitrógeno total (TN) de diferentes componentes vegetales de un BS. En este estudio se evaluaron dos especies de árboles nativos chilenos (*Drymis winteri* J.R. Forst. & G. Forst. y *Luma chequen* (Molina) A. Gray) y una pradera (*Festuca arundinaceae* Schreb.). El clorofilómetro CCM-200 y el Sistema Munsell de Color de Hoja (MSLC) fueron utilizados para estimar el TN en los árboles y la pradera, respectivamente. Para la DM de los árboles se evaluaron dos variables alométricas, diámetro basal de tronco (BD) y la altura (H). Se encontraron relaciones significativas (p<0.05) entre las lecturas de CCM-200 y las mediciones de TN en hojas de *D. winteri* y *L. chequen* con R^2_{adj} de 0.56 y 0.54 respectivamente. También se encontraron relaciones significativas (p<0.05) para la MSLC y el TN de *F. arundinaceae* (R^2_{adj}: 0.51). Sin embargo, los resultados sugieren que estos métodos no destructivos pueden sub o sobreestimar el contenido de N total debido a características intrínsecas de las hojas. En el desarrollo de las ecuaciones para estimar la DM, se encontró que solo BD presenta relaciones significativas (p<0.05) para predecir la DM de hojas, tallos y raíces de *D. winteri* y *L. chequen.* Para *D. winteri* el R^2_{adj} presentó valores entre 0,81 y 0,85 y *L. chequen* presentó R^2_{adj} de 0,15; 0,86 y 0,53 para hojas, tallos y raíces, respectivamente. Los resultados sugieren que las técnicas no destructivas son útiles para estimar la DM y el TN. Sin embargo, las predicciones de N presentan limitaciones asociadas con las características de las hojas influenciadas por factores ambientales y se hace necesario el desarrollo de ecuaciones de calibración más especificas.

Palabras clave: Ecuationes alométricas, medidor de contenido de clorofila, Sistema Munsell de Color de Hoja.

INTRODUCTION

Crop yield is affected by plant nitrogen (N) status. Thus, the optimization of N fertilization has become the object of intense research due to its environmental and economic impact (Muñoz *et al.*, 2013). A strategy for increasing the efficiency of N use and reducing the environmental impact of N surplus is the use of vegetative buffer strips (BS) (Ballestrini *et al.*, 2011; Mankin *et al.*, 2007). The BS remove N from water by different mechanisms, including denitrification, uptake by vegetation and soil microbes and retention in riparian soils. The uptake by vegetation is considered as an important source for reducing the N excess that will be entering to the water bodies, because of this, it is important to estimate the N excess that is retained in them. However, species specific N uptake values have not been studied much to date (Christen and Dalgaard, 2012).

Usually methods employed for N status determination in vegetal tissues, and thus to quantify the amount of N retained, are very effectiveness and accurate but at the same time are destructive and require sample pre-processing and analysis in specialized laboratories, which is high costly and time consuming (Da silva *et al.*, 2012). In consequence several researchers have focused on the design and application of non invasive methods for use in N status determination. Most of these methods have been developed using optical plant properties, which are affected by several factors: water content, leaf senescence, diseases, plant nutrients and plant N status (Muñoz *et al.*, 2013). For instance portable chlorophyll meters, such as SPAD-502 and CCM-200, have been developed based on transmittance properties of leaves. Both, SPAD and CCM, are based in transmittance of leaves exposed to two light sources: a red and an infrared light. The difference in transmission of the filtered wavelengths is the chlorophyll content indicator that can be closely correlated with leaf N status because this is an essential element of chlorophyll (Chang and Robinson, 2003; Girma *et al.*, 2006; Pinkard *et al.*, 2006). With portable chlorophyll meter therefore further simplifies quantifying N deficiency, making real-time, in the field diagnosis possible and allowing the promptly correct swards N deficiencies through fertilization (Errecar *et al.*, 2012).

The model CCM-200 of Opti-Sciences enterprises calculates CCI (Chlorophyll Content Index), as a ratio of transmittance at 935 nm and 635 nm from a specified leaf area of clamped leaf portion (Richardson *et al.*, 2002). Additionally, the model CCM-200, has showed positive correlations on different vegetal species (Errecar *et al.*, 2012; Van den Berg and Perkins, 2004), including tree species such as *Accer saccharum* (Cate and Perkins, 2003), *Rhizophora mangle* (Biber, 2007), *Pyrus serotina* (Ghasemi *et al.*, 2011), *Bombacopsis marocalyx, Eugenia cumini, Iryanthera macrophyla, Senna reticulate* (de Carvalho *et al.*, 2008), *Betula papyrifera* (Richardson *et al.*, 2002) and *Citrus* sp. (Jifon *et* *al.*, 2005). Thus compared to other chlorophyll meters, the CCM-200 have been successfully calibrated in tree species.

Other method to estimate chlorophyll status in vegetal tissues is the used of the Munsell System Leaf Colour (MSLC), which consider that the leaf colour intensity is related to leaf chlorophyll content and leaf N status (Islam *et al.*, 2007; Gomara, 2012). Some studies showed that the MSLC is a cost effective, simple and farmer’s friendly gadget to provide a guide to the based fertilizer N management in a broad range of crops (Maiti *et al.*, 2004; Singh *et al.,* 2011; Singh *et al.*, 2012). Both MSLC and CCM-200 can be used to determine the N level in a plant, thus it can be associated to the dry matter (DM) of the vegetal components and in this way estimate the total nitrogen (TN) content of the plant.

For measuring the DM of a plant it is necessary to harvest, where the total harvesting is generally impractical or inappropriate. An alternative way would be to use allometric methods that have been developed to estimate DM (Vann *et al.*, 1998). Allometrics methods consists in the evaluation of the change of proportion in some physiological or physical variable of an organism of easy measurement and non-destructive as a result of its growth (López *et al.*, 2003), such as crown area and relative height that can be correlated to a variable difficult to measure. These relationships inside a plant reflect the equilibrium between tree structure and their biomass (Porté *et al.*, 2002). Among the allometrics variables that have been successfully used to estimate the biomass showing a good agreement with the biomass are: i) the trunk diameter (Zhou *et al.*, 2007; Návar, 2009; Sampaio *et al.*, 2010); and ii) maximum height (Zhou *et al.*, 2007).

Therefore using non-destructive methods to determine the N status and DM of plants would be extremely valuable in establishing the N retention capacity of different plants. The main aim of this study was to develop different equations to estimate the DM and TN content of different vegetal components in a BS.

MATERIALS AND METHODS

Study sites

The experiments for calibration of non-destructive techniques were stablished in the Faculty of Agronomic Sciences at the University of Chile, Commune of La Pintana, Santiago, Chile. Chemical analyses were carried out at the Soil and Water Chemistry Laboratory located in the same Faculty.

Plant material

Two native tree species were considered: *Luma chequen* (Molina) A. Gray or “chequén” and *Drymis winteri* J. R Forst & G. Forster or “canelo”; and a grass specie fescue (*Festuca arundinacea* Schreb.). The *L. chequen* and *D. winteri* were obtained at the plant nursery at the Faculty of Forestry Sciences and Nature Conservation, whereas for *F. arundinaceae* seeds of a tall variety (faw) were used.

Greenhouse experiment

Three greenhouse essays were carried out, including trees (Experiments 1 and 2) and the grasses (Experiment 3).

Experiment 1. Ten *L. chequen* and ten *D. winteri* trees were planted: of at least 2 years old, about 40 cm tall and without fertilization. The experiment was established in January 2013. Soil belongs to the Santiago soil Series.

Experiment 2: Eight *L. chequen* and eight *D. winteri* trees were planted: of at least 2 years old and about 40 cm tall. However, increasing doses of N-urea was applied (0, 100, 200 and 300 kg N ha^{-1}). These plants were established in sixteen containers with soil belongs to the Santiago soil Series between September 2014 and January 2015.

Experiment 3: sixteen pots were seeded with *F. arundinacea* considering a sown rate of 20 kg ha^{-1} (Ovalle *et al.* (2011). Increasing doses of N-urea was applied (0, 100, 200 and 300 kg N ha^{-1}) in the pots, considering four replication per doses. The fescue was sown in June 2012 and harvested in January 2013 and soil at pots belongs to the San Luis soil Series.

Plant measurements

Total nitrogen content measurement

Plants were harvested and their different structures were separated in leaves, stems and roots for trees, but only in leaves and roots for *F. arundinacea*. All the vegetal samples were separately dried at 70°C until constant mass and analyzed in laboratory to measure the TN content according to Sadzawka *et al*. (2007). This methodology considered 0.5 g of sample sieved at 0.5 mm, which is subjected to acid digestion followed by steam distillation.

Chlorophyll and Munsell colour measurements

Two methods were used for plant measurements: i) for the tree species a hand-held chlorophyll meters (Opti-science, model CCM-200) and ii) for the fescue the Munsell System Leaf Colour (MSLC). The CCM-200 uses differential transmission at two wavelengths, 660 and 940 nm, calculating a chlorophyll content index (CCI) based on absorbance. In the measurements, major veins and areas of obvious visual damage or disease were avoided. In *D. winteri* five leaves were collected and six readings with CCM-200 were done in each leaf. The measurement of *L. chequen* was done in five branches with at least six leaves, where a single CCI reading was taken in six leaves of the branches. For each tree species eighteen plants were evaluated.

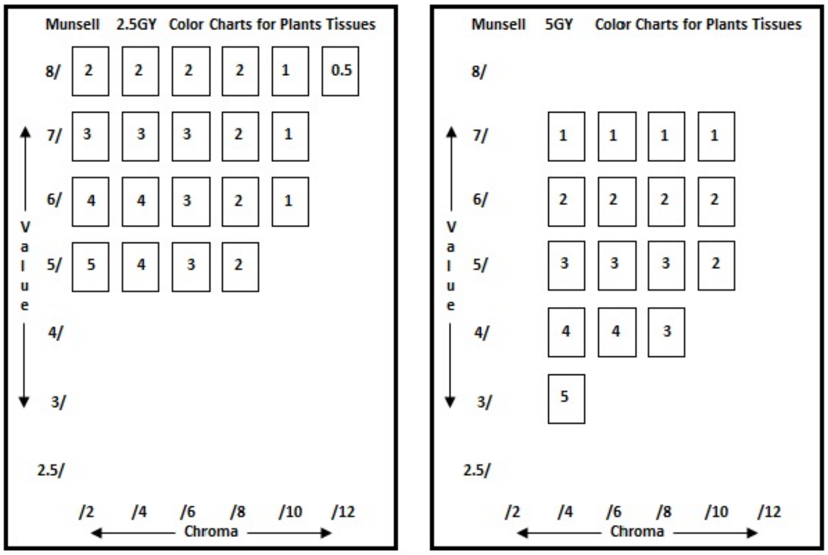

Figure 1. Relative numerical scale for Munsell colour cells, hue 2.5GY (left) and hue 5GY (right).

The MSLC describes the colour in terms of three attributes called: hue, value and chroma. The colour was measurement in leaves of *F. arundinacea* from the experiment 3, but previously each color cell was converted to a relative numerical scale. So that, an Arabic number (Munsell number or MN) was assigned to each color cell (Figure 1). The MN rangerd from 0.5 to 5.0 in the different hues, where 0.5 correspond to the colours with the highest chroma and value, and 5.0 to colours with the lowest chroma and value.

These measurements were associated statistically with the measured TN contents of the plants.

Growth parameters and dry matter

Allometric variables were also measured (tapes and vernier caliper) at both tree species, i.e., basal trunk diameter 10 cm above the soil surface (BD) and height (H). The utilization of non-destructive allometrics methods consists in the evaluation of some physiological or physical characteristics of an organism to predict their future behaviour, which are easy to measure (López *et al.*, 2003). These relationships inside a plant reflect the equilibrium between tree structure and plant growth or biomass (Porté *et al.*, 2002).

The *D. winteri*, *L. chequen* and *F. arundinacea* were harvested and separated in leaves, stems and roots, as the case may, and put in paper bags, after it, were submitted to drying at 70°C for 48 hours until constant mass. Subsequently all the bags were weighed to determine the dry matter (DM) of the different structures.

Statistical analyses

The results of TN content, measurements of CCI and MSLC for the different species, were analysed using correlation coefficient and then by a simple regressions to determine the relationship between these parameters, considering the calculation of the adjusted coefficient of determination (R^2_{adj}). Using simple regression were developed equations that correlate the TN concentration and CCI or MV.

In the case of the results obtained of DM and allometric variables in trees, there were developed multiple regression models for explaining the variation in DM, where a backward selection procedure was performed by deleting predictors from the model considering the *p*-value of the evaluated variables.

All the previous statistical analysis was performed using InfoStat statistical program.

RESULTS AND DISCUSSION

Chlorophyll content index versus total nitrogen content

Means values for TN content of different structures of *D. winteri*, *L. chequen* and *F. arundinacea* are shown in Table 1. These values for *D. winteri* leaves are higher than the measured by González *et al*. (1990), who reported mean values from 0.9 to 1.5 % in adult *D. winteri* in the south of Chile. However, studies (van den Berg and Perkins, 2004; Ghasemi *et al*., 2011; Da Silva *et al*., 2012) performed in other tree species report values of TN content in leaves similar to the results of *D. winteri* and *L. chequen*. Considering the mean value in N content for the different structure of both three species, *D. winteri* would be more suitable for riparian zone owing to higher N removal from the soil. However, because of the different values of N on the components of each species it is important to stablish these for improved estimates of N storage in tree biomass (Peichl *et al*., 2012).

Table 1. Means values for total nitrogen content of different structures of canelo (*Drimys winteri* Forst), chequén (*Luma Chequen* (Mol.) A. Gray) and fescue (*Festuca arundinacea* Schreb.) plants.

	Total nitrogen		
Plant structure	*D. winteri*	*L. chequen*	*F. arundinacea*
	------------------------ % --------------------------		
Leaves	2.57 ± 0.57	1.69 ± 0.43	1.47 ± 0.65
Stem	1.04 ± 0.20	0.87 ± 0.17	---
Roots	1.13 ± 0.30	1.04 ± 0.17	---

The CCI in the 18 plants of *D. winteri* ranged from 14.6 to 37.8 with a mean of 31.3 ± 5.4. Correlation coefficient between CCI and TN content in leaves, and between stem and roots are shown in Table 2. Although for *D. winteri* the correlation coefficient of leaves with CCI showed a significant correlation, for stem and roots did not show a good agreement. On the other hand, measured with CCM-200 in *L. chequen* ranged between 9.3 and 70.4 with a mean value of 33.5 ± 16.0, showing a good agreement between different structures evaluated and the CCI measurements. Clearly the best relationships were found between TN content of leaves and CCI for both species.

Table 2. Pearson correlation coefficient between Chlorophyll Content Index and total Nitrogen of different structures in canelo (*Drimys winteri* Forst) and chequén (*Luma chequen* (Mol.) A. Gray) and between Munsell number and total Nitrogen in fescue (*Festuca arundinacea* Schreb.).

Species	*D.winteri*	*L. chequen*	*F. arundinacea*
Leaves	0.73	0.76	0.73
Stem	0.03	0.46	---
Roots	-0.65	0.29	---

The relationship between CCI and TN content in leaves was different between *D. winteri* and *L. chequen*; whereas *D. winteri* present a significantly non-linear relationship (*p*-value < 0,05), in *L. chequen* found a significantly linear relationship (*p*-value <0,05) with a R^2_{adj} of 0.56 and 0.54 for *D. winteri* and *L. chequen*, respectively. Other authors found significantly agreements between CCI and N content in leaves. For example, Jifon *et al.* (2005) showed R^2_{adj} values of 0.29, to 0.69 in different cultivars of *Citrus* sp. Also good relationship was found in Pyrus (*Pyrus serotina* Rehd.) and sugar maple (*Acer saccharum* Marsh.) with R^2_{adj} ranging between 0.64 and 0.76 (van den Berg and Perkins, 2004; Ghasemi *et al*., 2011).

Using regression analysis there were developed equations for *D. winteri* and *L. chequen* trees that relate the CCI with TN content (see Figures 2 and 3). The equations 1 and 2 are for *D.winteri* and *L. chequen*, respectively:

$$TN(\%) = 0.850e^{0.034CCI} \qquad \text{(Eq. 1)}$$

$$TN(\%) = 0.020CCI + 1.012 \qquad \text{(Eq. 2)}$$

The behaviour of CCM-200 equipment has been previously studied by several authors for development of prediction equations for chlorophyll (Chl) and N status in plant. For instance, Richardson *et al*. (2002) reported a non-linear relationships between CCI and Chl for paper birch and Pal *et al*. (2012) for damask rose, but other researchers have also found a linear relationship (Cate and Perkins, 2003; Jifon *et al.*, 2005). Similarly occurs when it is compared CCI with N status, when Van den Berg and Perkins (2004) found linear relationship in sugar maple, whereas Pal *et al.* (2012) found that the best prediction equation was a power relationship for damask rose. Clearly, relationship between Chl, N and CCI do not exhibit similar patterns for all species when this relationship not only depends of the species, but also of the growing conditions. The above may be related to the difference between species and as stated growing condition, such as different level of fertilizer that modified the Chl a/b ratio, size of chloroplasts or distribution of these (Bondada and Syvertsen, 2003) affecting the transmission of light though the leaf.

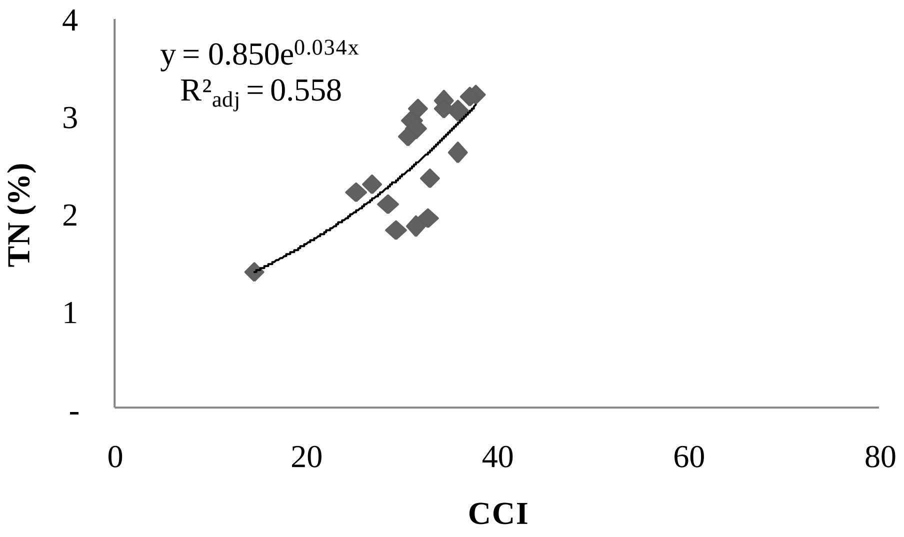

Figure 2. Non-linear regression of chlorophyll content index (CCI) versus total nitrogen (TN) content in leaves of *D. winteri* (n = 18).

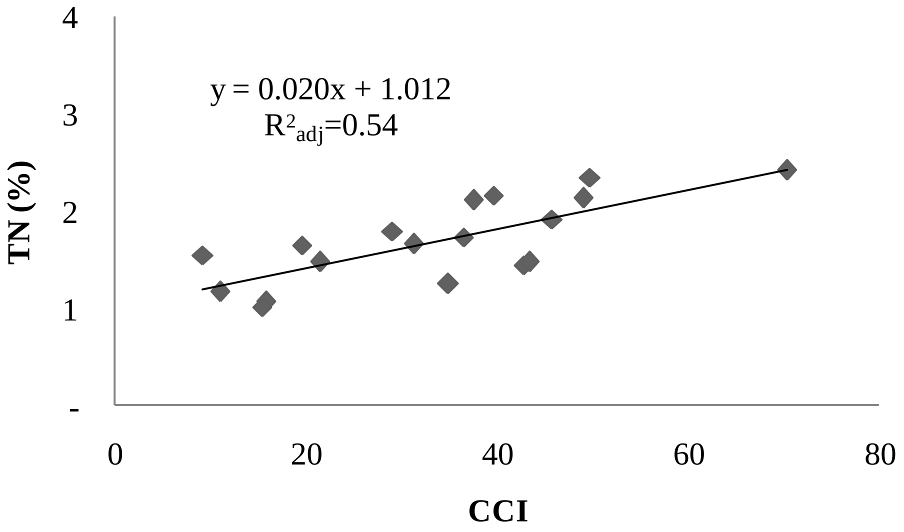

Figure 3. Linear regression of chlorophyll content index (CCI) versus total nitrogen (TN) content in leaves of *L. chequen* (n = 18).

In *L. chequen* there was found a best agreement between TN content y CCI with a linear model (R_{adj}= 0.54), whereas *D. winteri* had the best agreement between TN content y CCI (R_{adj}= 0.56) with an non-linear model. For *D. winteri* the distribution of the model space for CCI measurements ranged from 15 to 40 and for TN content from 1.5 to 3.5%, whereas for *L. chequen* the distribution of the model space for CCI measurements ranged from 10 to 70 and for TN content from 1 to 2.5%.

It is clear that the range of CCI measurements is restricted, when the clumping of the CCI measurement can be related to the saturation of the Chl meter. Considering that the TN content is linked with amount of Chl in the leaf and the operation of CCM-200 use the transmittance of red and infrared light, thus the CCI measurements can be affected by the system of chloroplasts and the distribution of the Chl in the leaf. Parry *et al* (2014) highlighted that if Chl is uniformly distributed the CCI values would be related to Chl concentration as a logarithmic function. However, it is not uniformly distributed in leaves and these can causes the decreases transmission of light at lower Chl concentration and increases transmission of light at higher Chl concentrations. In consequence CCM-200 equipment can under or overestimate the value of CCI causing the detour and sieve effects. The detour effect increases the optical path-length through the leaf, which reduces light transmission while the sieve effect causes transmission to increase and thus the optical chlorophyll measurement is lower than a sample with uniform Chl distribution (Richardson *et al.,* 2002; Parry *et al.*, 2014). The measured high N values in *D. winteri* can be affected by these effects, considering that high level of N in leaves is associated with high levels of Chl, but these increased the Chl density in chloroplast not the number and rearrangement of the chloroplast with a reduction of light absorption (Pal *et al.*, 2012). In addition, the distribution of Chl in leaves is uniform with low Chl leaves (Uddling *et al.,* 2007). Although with high level of N it is more probable that the sieve effect occurs and underestimate the values of CCI, the effectiveness of CCM-200 is lower at high concentrations of Chl. The same effects are reported by Pal *et al.* (2012) for CCM-200 that underestimated total Chl at high concentrations.

Although a high N content it translates to a high Chl content, it is not always true. For Pal *et al.* (2012) more than 80% of the variation in N was predicted by CCM-200 readings and concludes that is probably due to the fact that more N is bound up in Chl and other photosynthetic compounds. Although, N can be component of others structures such as protein, DNA or founded soluble in leaves, the measuring of the same CCI values with a high and low N concentration can be underestimate or overestimate the TN content, because CCI is an indirect method to estimateTN content. Therefore, can be the reason of the low R^2 for both *L. chequen* and *D. winteri* equations. The uneven distribution of N between soluble proteins and the light-harvesting complex can be intensified by leaf age, growth environment or cultural practices (Bondada and Syvertsen, 2003). This is related to the fact that Chl concentration can have significant spatial variation it is important as proposed Parry *et al.* (2014) to remove the leaf disk, for N extraction, from exactly the same location as the optical measurement.

Munsell number versus total nitrogen content

The MSLC was used to determine the colour for leaves of fescue in the experiment 3, but also its necessary agree some colours found in plants localised in the field. This measurement showed a variety of readings with a total of 12 different colours, for each colour was assigned the corresponding MN (See Appendix I). The results of the TN content ranged from 0.76 to 3.58 %, with a mean value of 1.47%. The literature is variable about the value of TN content in *F. arundinacea*. A study reported TN content for a grazing system, where a present species is fescue. In this system, the N concentration ranged between 2.47 and 2.90 % (Nuñez *et al.*, 2010). Roberts *et al.* (1988) found values ranging from 0.70 to 2.91 %, similar to the results of present experiment. However, Bake *et al.* (2000) considered 2.50 % as the critical value of TN content in fescue.

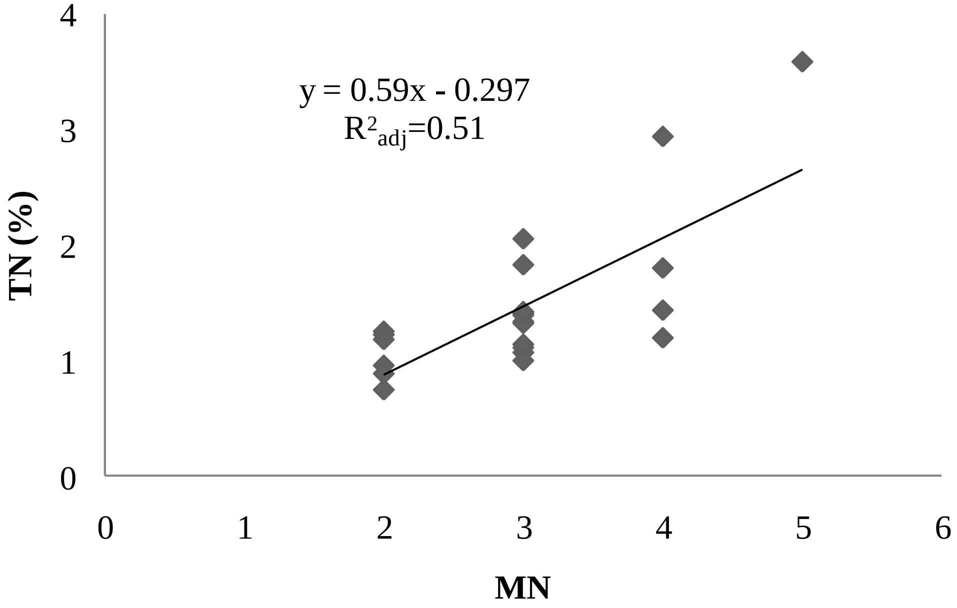

Figure 4. Linear regression of Munsell number (MN) versus total nitrogen (TN) content in leaves by dry weight of fescue (*Festuca arundinacea* Schreb.) (n=23).

The correlation between MN and TN content is shown in Table 2; this coefficient is good for TN content in leaves of fescue. The relationships of these two variables are significantly linear (p-value< 0.05) and present an R^2_{adj} of 0.51(Figure 4). The results of the linear regressions are included in Equation 3:

$$\text{TN (\%)} = 0.59\ \text{MN} - 0.297 \qquad \text{(Eq. 3)}$$

Growth parameters versus dry matter

For *D. winteri* BD ranged from 0.5 to 1.66 cm, the other allometric parameter ranged from 24.4 to 97.0 cm. In the case of the DM the results showed that the DM above ground corresponds generally to the half of the total DM of *D. winteri*. The values for DM of leaves, stem and roots ranged from 1.09 to 40.96 g, 0.43 to 33.54 g and 1.84 to 56.51 g, respectively. In the case of *L. chequen* the allometric parameters ranged between 0.35 to 1.19 cm and 48 to 88 cm for BD and H, respectively. The results of DM of leaves, stem and roots were ranging from 1.92 to 28.62 g, 3.47 to 17.93 g and 2.31 to 20.87 g, respectively. The DM above ground on average was three quarters of the total DM. In Table 3 are shown mean values of the variables.

Table 3. Basal trunk diameter (BD), height (H) and dry matter of different structures of canelo (*Drimys winteri* Forst) and chequén (*Luma chequen* (Mol.) A. Gray).

Tree specie	BD	H	Leaves	Stem	Roots
	---------- cm ----------		----------------- g -------------------		
D. winteri	0.83 ± 0.38	50.12 ± 28.44	11.54 ± 13.79	8.70 ± 10.66	16.68 ± 20.69
L. chequen	0.67 ± 0.32	63.63 ± 11.05	7.04 ± 6.18	8.82 ± 4.48	6.66 ± 4.92

Considering the previous results, the correlation coefficient between the different structures and the allometric parameters are shown in the Table 4. In most of the structures was found a good correlation coefficient with the allometric variables with values above 0.6. In general, there is good correlation between the measured parameters. However, *L. chequen* showed the highest variability in the results, when it was found a correlation coefficient between leaves and H lower than 0.1.

In addition, a multiple regressions analysis was carried out for *L. chequen* and *D. winteri* with the DM of the different structure. In Table 5 are shown the results of the multiple regression, considering the *p*-value of the variables.

Table 4. Pearson correlation coefficient of the dry matter of different structures of Canelo (*Drimys winteri* Forst) and Chequén (*Luma chequen* (Mol.) A. Gray) with the allometric parameters basal trunk diameter 10 cm above soil surface (BD) and height (H).

Parameter	*D. winteri*			*L. chequen*		
	Leaves	Stem	Roots	Leaves	Stem	Roots
BD	0.92	0.93	0.90	0.44	0.93	0.75
H	0.87	0.88	0.87	0.04	0.62	0.47

Most of the models developed with the multiple regression was significant (*p*-value < 0.05), except the model for the leaves of *L. chequen* (*p*-value 0.07) (see Table 5). The *D.*

winteri R^2_{adj} presented values of 0.84, 0.86 and 0.82 for leaves, stem and roots, respectively. In *L. chequen* there were found values of 0.2, 0.85 and 0.5 for leaves, stem and roots, respectively. Although the models were significant ($p<0.05$), the H was not significant ($p > 0.05$) in all the models, thus the H was discarded and only BD was considered to development a model that explains the growth of *D. winteri* and *L. chequen*.

Table 5. Statistical significance of the multiple regression models for Canelo (*Drimys winteri Forst*) and Chequén (*Luma chequen* (Mol.) A. Gray).

Parameter	*D. winteri*			*L. chequen*		
	Leaves	Stem	Roots	Leaves	Stem	Roots
Model	<0.0001	<0.0001	<0.0001	0.0703	<0.0001	0.0022
H	0.2554	0.1577	0.1542	0.1613	0.7594	0.9285
BD	0.0035	0.0032	0.0143	0.0236	<0.0001	0.0039

Considering BD was carry out simple regression models for the structures of *D. winteri* and *L. chequen*. The results of these regressions are shown in Figure 5. In the case of *D. winteri* were developed the equations (4), (5) and (6) for leaves, stem and roots, respectively:

$$DM_{leaves} = 33.81\ BD - 16.40 \quad \text{(Eq. 4)}$$

$$DM_{stems} = 26.28\ BD - 13.01 \quad \text{(Eq. 5)}$$

$$DM_{roots} = 49.70\ BD - 24.39 \quad \text{(Eq. 6)}$$

The equations that were developed for *L. chequen* presented a more variable behaviour and the lowest R^2_{adj} values, where the equations (7), (8) and (9) were developed for leaves, stem and roots, respectively:

$$DM_{leaves} = 8.631\ BD + 1.275 \quad \text{(Eq. 7)}$$

$$DM_{stems} = 13.17\ BD + 0.018 \quad \text{(Eq. 8)}$$

$$DM_{roots} = 11.61\ BD - 1.092 \quad \text{(Eq. 9)}$$

Regression equations are developed to estimate above ground biomass of foliage and stem size fractions from plant size dimensions. For instance McClaran *et al.* (2013) found significant equations with R^2_{adj} values >0.72 for BD, H, canopy area and total leaf area. In our results, H did not show a good agreement with DM. However, H has been one of the more used allometric variables in trees by different authors (McClaran *et al.*, 2013; McNicol *et al.*, 2015; Zhou *et al.*, 2007). The BD has been usually included in allometric equations, and in our study for *D. winteri* and *L. chequen* presented a good agreement with DM. The R^2_{adj} of the different equations is higher than 0.8, except in the case of the leaves and roots of chequén, where the results showed values of 0.15 and 0.53, respectively. Other authors found strong relationships between BD and tree components, for example: Peichl e*t al.* (2012) reported values of R^2_{adj} between 0.9 and 0.98 for different components of ash and alder tree; Northup *et al*. (2005) found values between 0.69 to 0.96 for leaves and stem of subtropical woody species; Vann *et al.* (1998) in *P. uviferum* and *F. cupressoides* also reported strong relationship between BD and DM of different tree components. Although these authors found higher values of R^2_{adj} than the reported in our study, these authors considered a larger amount of trees and a wider age range.

Other studies considered only the above ground DM as allometric variable (Porté *et al.*, 2002, Northup *et al*., 2005). However, we found good relationship within the BD and the root DM. For *D. winteri* and *L. chequen* the R^2_{adj} was 0.81 and 0.53, respectively. Therefore, the BD could be a good predictor of the DM of roots in trees. As well, McNicol *et al*. (2015) development an allometric model for those roots that considered only the BD.

Northup *et al.* (2005) considered that metrics based on BD was more robust due to it was less sensitive to short-term environmental fluctuations. However, it is important to note that the robust of the allometric relationships may be or the degree to which they may vary with soil type, geomorphology, land use or disturbance history. Further equations have been developed across sites and species in woodland ecosystems, but within species variation in architecture has even documented variation along landform gradients (McClaran e*t al.*, 2013). Therefore the generation of allometric equations have to consider a number of variables, and it is would be more difficult to develop for native species when there is lack of information.

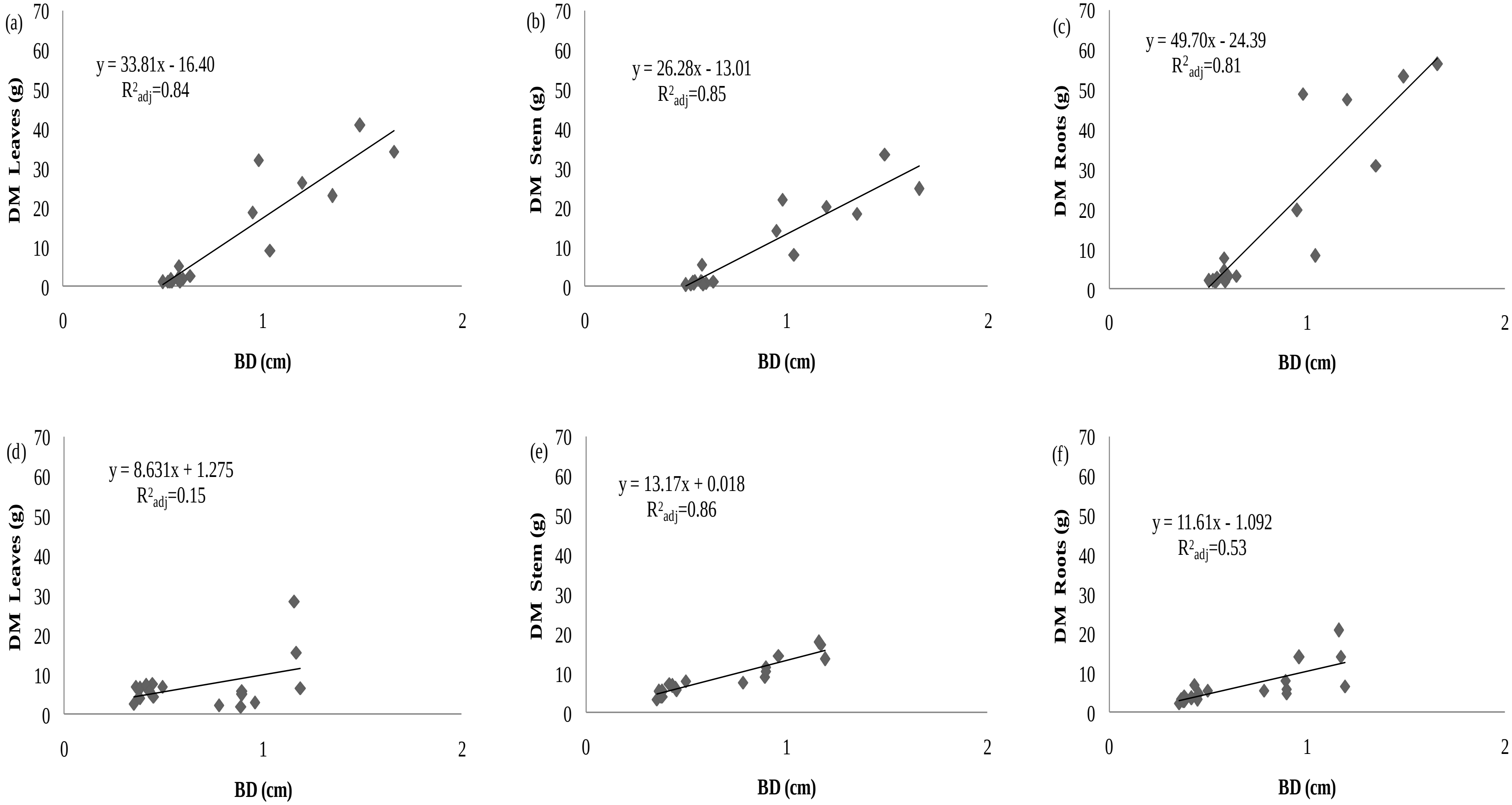

Figure 5. Linear regression for *D. winteri* (a, b and c) and *L. chequen* (d, e and f) between basal trunk diameter 10 cm above the soil surface (BD) and dry matter (DM) of leaves, stem and roots.

CONCLUSIONS

The use of portable equipments, such as CCM-200 and MSLC, provide a rapid and easy estimation of the chlorophyll content that can be related to the total nitrogen (TN) content. For *D. winteri*, *L. chequen* and *F. arundinacea* significant relationships were found between the equipment readings and N concentration in leaves, which can be used for prediction of TN content without the destruction of plant. However, considering the results of both techniques, these can over or underestimate the TN content of leaves. Particularly with CCM-200 the difference in the morphology of the leaves on different conditions of growing and the distribution of the N in leaves make necessary a better calibration of the equipment. On the other hand, the development of allometric equations is an important tool to conduct studies without the intervention of the system to be analized, especially when studied species are in danger of preservation. In this case, it was possible to successfully establish equations to estimate the total biomass of native species *D. winteri* and *L. chequen*. Basal trunk diameter (BD) and height (H) were evaluated as allometric variables, although the H was not effective as expected, the use of BD was satisfactory to establish equations to explain the dry matter for both species. However, it is possible that H or other allometric variables can be better adapted to older plants or other growth conditions, so its use should not be ruled out.

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CHAPTER III: APPLICATION OF NON-DESTRUCTIVE TECHNIQUES TO ESTIMATE THE NITROGEN RETENTION IN THE VEGETAL COMPONENTS OF BUFFER STRIPS

ABSTRACT

The nitrogen (N) water pollution is a global issue, and agriculture is one of its main causes. The over fertilization of crop with N and wrong time and place of N applications may generate N losses from soils to nearby water bodies. One of the most widely approaches used for N pollution mitigation in cultivated soils are the vegetative buffer strips (BS). In this study, a BS (5 m wide) with three different vegetal covers were established and their N retention capacity was compared. The treatments correspond to a combination of two Chilean native trees species (*Drymis winteri* J.R. Forst. & G. Forst. y *Luma chequen* (Molina) A. Gray) and a prairie (*Festuca arundinaceae* Schreb.), with tree replication per treatment with a randomized block design (Blocks 1-3) distributed in 5 m x 12 m plots. The field experiment was set in two maize cultivated soils named El Caleuche (CLC) and San Luis (SL) in 2012, in the Pichidegua commune, O'Higgins Region, which present contrast textural classes such as clay and sandy in CLC and SL, respectively. For the estimation of the total N retention of the BS was considered the dry matter (DM) biomass and the N content for each vegetation component, which were measured by non-destructive methods. In CLC there were not significantly differences (*p*-value>0.05) in the N retention among treatments, whereas in SL the BS covered by permanent grass (G treatment) showed significantly (*p*-value<0.05) higher N retention than the treatments with trees (GST1 and GST2). The prairie was the principal component for N retention in all the BS. The treatments did not show significant differences (*p*-value>0.05) between sites. N uptake by grass was an important process in the N retention capacity of the BS, whereas young trees and shrubs showed limited N uptake in the same period.

Keywords: Dry matter biomass, tree component and prairie.

APLICACIÓN DE TÉCNICAS NO DESTRUCTIVAS PARA ESTIMAR LA RETENCIÓN DE NITRÓGENO EN LOS COMPONENTES VEGETALES DE UN BIOFILTRO

RESUMEN

La contaminación de aguas por nitrógeno (N) es un problema mundial y la agricultura es uno de sus principales causas. La sobre fertilización nitrogenada de cultivos y el momento y lugar equivocado de las aplicaciones de N pueden generar pérdidas de N desde los suelos hacía cuerpos de agua cercanos. Una de las medidas más ampliamente utilizados para la mitigación de la contaminación por N son los biofiltros (BS). En este estudio, un BS (5 m de ancho) con tres cubiertas vegetales diferentes fue establecido, donde se comparó su capacidad para retener N. Los tratamientos correspondieron a una combinación de dos árboles nativos chilenos (*Drymis winteri* J.R. Forst. & G. Forst. y *Luma chequen* (Molina) A. Gray) y una pradera (*Festuca arundinaceae* Schreb.) con tres repeticones por tratamiento en un diseño de bloques completamente aleatorizados (bloques 1-3) distribuidos en parcelas de 5 m x 12 m. El experimento de campo se estableció en dos suelos cultivados con maíz llamados El Caleuche (CLC) y San Luis (SL) en el año 2012, en la Comuna de Pichidegua, Región de O'Higgins, estos presentan clases texturales contrastantes tal como arcillosa y arenosa en CLC y SL, respectivamente. Para la estimación de la retención total de N del BS fue considerada la materia seca total (DM) y el contenido de N de cada componente vegetal, los que fueron medidos con métodos no destructivos. En CLC no hubo diferencias significativas (*p*-value>0.05) en la retención de N, mientras que en SL el BS cubierto con una pradera permanentemente (tratamiento G) que mostró una retención de N significativamente (*p*-value>0.05) mayor que los tratamientos con árboles (GST1 y GST2). La pradera fue el componente principal en la retención de N de todos los BS. Los tratamientos no mostraron diferencias significativas (*p*-value>0.05) entre los sitios. La absorción de N por la pradera fue un proceso importante en la capacidad de retención de N de los BS, mientras que los árboles jóvenes mostraron una limitada absorción de N en el mismo período.

Palabras clave: Materia seca total, componente arbóreo y pradera.

INTRODUCTION

The intensive use of fertilizers and organic fertilizers to increase food production can increase the risk of contaminating water bodies with nitrogen (N). This element in excess, when is transported to surface water bodies, create serious problems of eutrophication in coastal areas around the world and contaminates groundwater (Carpenter *et al.*, 1998). Additionally, the consumption of water with high levels of nitrate (NO_3^-) generates various adverse effects on the health of animals and humans (Ward *et al.*, 2005). Currently, diffuse sources of pollution, are the primary sources of pollution of water bodies and the main contributor is the agriculture (Dowd *et al*., 2008). Therefore, agricultural areas that found around water bodies are considered more prone to loss N via leaching or runoff (Vitousek *et al.*, 1997; Stålnacke *et al.*, 1999). Riparian vegetation has a critical role to play in non-point source pollution abatement and water quality protection within watersheds in agricultural areas (Fortier *et al*., 2010).

One of the best management practices that have been used for retaining nutrients and sediments from leaching or surface runoff are vegetative filter strip or (BS). This mitigation measure prevents the pollutants from reaching receiving waters (Bhattarai *et al.*, 2009). BS are strips of land with permanent vegetation, usually shrubs and meadows, designed to intercept surface runoff and subsurface lateral flow, to reduce the content of N transported by water (Mayer *et al*., 2006). In addition, soil microbes and vegetation, can facilitate this transformation and absorption of dissolved N that is moving.

In the last 20 years, there have been a number of studies in different countries of the world that have evaluated the effectiveness of the biofilters in retaining N (Burt and Haycock, 1993; Chaubey *et al*., 1994; Borin and Bigon, 2002; Lin *et al.*, 2002; Borin *et al.*, 2005; Hefting *et al.*, 2005; Mihara *et al.*, 2005; Rassam *et al.*, 2006; Syversen, 2005, Van Beek *et al.*, 2007; Duchemin and Hoguen 2009; Fortier *et al.*, 2010; Webber *et al.*, 2010; Balestrini *et al.*, 2011; Dunn *et al.*, 2011; Wang *et al.*, 2012). In these studies have been noted that there are multiple factors involved in the effectiveness of BS to reduce N losses to surface water courses; among them are the most crucial when implementing this mitigation management: the hydrological characteristics of the area, the width and plant composition of the BS.

Thus has been done extensive research on the use of BS in agricultural soils, finding that the width of the BS could be positively related to their effectiveness in removing N (Grismer *et al.*, 2006). Even results indicate that BS of 5-8 m wide present high effectiveness in removing N (Borin and Bigon, 2002; Borin *et al.*, 2005; Balestrini *et al.*, 2011). Regarding plant composition their retention efficiencies are determined by many factors, among others the type of plant species, its growth stage and morphology

(Lambrechts *et al*., 2014). In the BS it is possible to combine three vegetational strata: prairie, shrub and trees, each one with defined characteristics (Tapia y Villavicencio, 2007).

On the one hand, the grass strip component slows and spreads runoff, filters sediment, resists erosion by rill flow common in row crops, takes up nutrients during the growing season and furthers denitrification. It can be used to produce energy grasses, hay and silage, pasture for extensive grazing and biogas feedstock (Christen and Dalgaard, 2012). On the other hand, shrubs and trees provides the best conditions for infiltration of runoff and thereby retention of suspended sediment particles and removal of dissolved pollutants, immobilizes nutrients in woody biomass and greatly enhances denitrification potential. Production options are woodchips firewood, pulpwood and in case of more careful long-time management also sawlogs for parquet, furniture and special uses (Christen and Dalgaard, 2012).

Despite the many studies that have been conducted, few have focused on the N uptake capacity of the plants and the composition of the BS. Even it has even been said that the plant composition and N uptake capacity would not be relevant to the BS system (Mayer *et al*, 2006). In this case, nutrients taken up by plants would remain in the system only temporarily and may be gradually released by mineralization later. Still, plants increase the residence time of nutrients considerably by reducing their mobility. However, Fortier *et al*. (2015) comparing poplars buffers with herbaceous buffers conclude that the first can stored 9-31 times more carbon biomass and 4-10 more N biomass. Other studied conduced in Pichidegua estimates than the combination of prairie with eucalyptus could achieve an N uptake of 720 kg ha^{-1} yr^{-1} (Tapia y Villavicencio, 2007).

Therefore, it is necessary to continue looking for plants suitable to the specific conditions of the area, where the BS want to be implemented and maximizes the N retention capacity of the BS.

The main objective of this study was evaluate the effectiveness in N retention of BS considering its plant composition and its different combinations.

MATERIALS AND METHODS

Study sites

The study was conducted in the Libertador General Bernardo O'Higgins region (Central zone of Chile), in two sites located in the province of Cachapoal, commune of Pichidegua.

The sites are under a system of monoculture maize for grain during spring-summer period (september to april), with a fallow during autumn-winter period (may to august). During the crop season the soil is irrigated by a furrow irrigation system. The sites were identified, according to the locality in which they are, as San Luis (SL) (34 ° 22' S , 71 ° 25'O and 124 m) and the Caleuche (CLC) (34 ° 25 ' S , 71 ° 21'O and 136 m), which are adjacent to surface water bodies (Figure 6). The soil in CLC belongs to the El Caleuche soil Serie, which is a fine-loam surface textural class, nearly level, presents a duripan in deep and is classified as Typic Duraqualf; whereas, the soil in SL is included in undifferentiated alluvial terraces with sandy loam surface textural class, moderately deep, nearly level, with excessive internal drainage and occasionally flooded and classified as Typic Xerept.

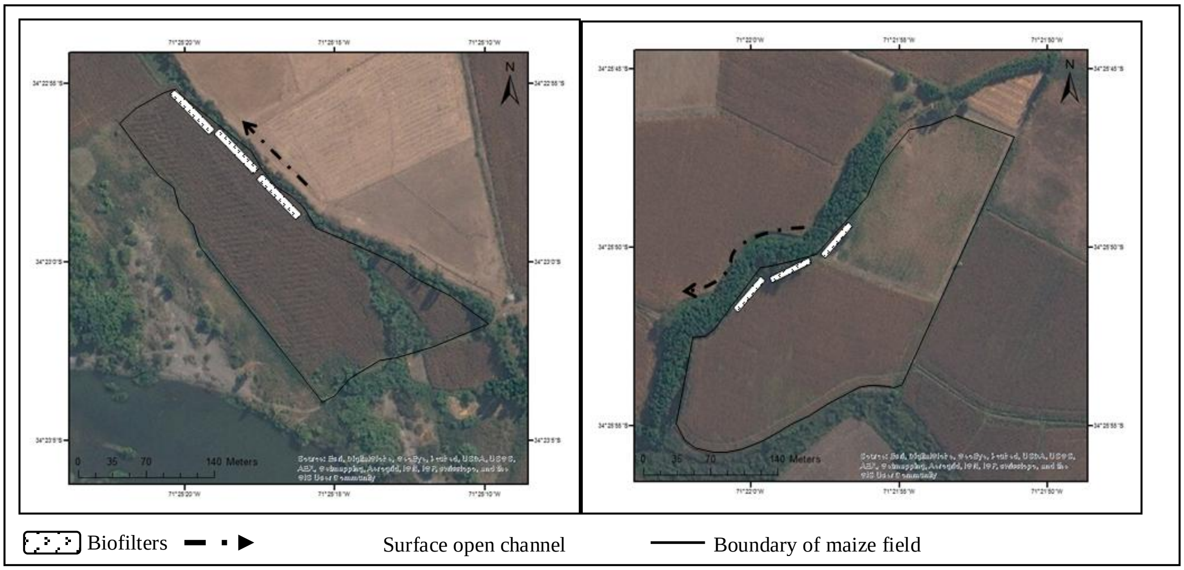

Figure 6. Satelital images of study sites San Luis (left) and Caleuche (right). The white rectangle indicates the position of the buffer strips (blocks), whereas the arrow indicates the course of surface water and its flow direction.

The climate in the study area was classified as temperate with dry, warm summers and relatively cold winters, corresponding to Csb according to the Köppen-Geiger System (Peel *et al.,* 2007). Mean annual temperature at the site is 14.1 °C and mean annual precipitation around 490 mm, mostly falling between May and October (INIA, 1989). The rainfall distribution is strongly seasonal, with 75 % falling in the autumn-winter period (April-September).

Treatments and experimental design

For both sampling sites were considered three treatments (Table 6), with three repetitions of each one in a total of nine experimental units (plots). The experimental design corresponded to a randomized block design (Blocks 1-3). To set the vegetal composition of the BS, two Chilean native trees were selected, able to tolerate prolonged periods of flooding; these species were: chequén (*Luma chequen* (Mol) A. Gray.) and canelo (*Drimys winteri* JR et G. Forster). The tree components was planted in 2012 and was necessary replanting in 2013 due to the dead of some trees. Also was established fescue (*Festuca arundinacea* Schreb.) as prairie component of the BS, *F. arundinacea* was seeding during July and August 2012 considering a seeding rate of 20 kg ha^{-1}.

The sites had abundant maize stover and weeds and were cleaned before planting, which was carried out in May 2012. In both sites, the BS were established perpendicular to the dominant slope.

Table 6. Description of the treatments.

Treatment	Species	Description
Grass (G)	Fescue	A strip of grass (5 m wide)
Grass + tree 1 (GST1)	Fescue + *L. chequen*	A strip of grass (3 m wide), and a row of trees (6 trees, 2 m wide planting)
Grass + tree 2 (GST2)	Fescue + *D. winteri*	A strip of grass (3 m wide), and a row of trees (6 trees, 2 m wide planting)

Methodology

The study was carried out from May 2012 to July 2014. In each treatment was calculated of the total N retention using the equations presented in Chapter II.

Plant measurement

The basal trunk diameter (BD), height (H) and CCI of the native trees *D. winteri* and *L. chequen* was measured. These measurements were carried out considering the procedure described in Chapter II. Four measurements were made during August 2013 and July 2014. For the leaf colour of the prairie was used the MLCC, in the same way of the procedure described in Chapter II. The colour of the prairie was measured three times during the study. The measurements of colour, BD, H and CCI were considered to determine the total dry matter (DM) and total nitrogen (TN) content of the trees.

Dry matter of the prairie

To determine N prairie uptake, plant tissue samples were collected during the essay using a point quadrat (0.25 m^2) with three replicates per treatment. In total, three samplings were carried out during the study period. These plant samples were used to determine the DM at 70°C.

Determination of nitrogen retention

To determine the N retention in the tree species, the measurements of BD, H and CCI were considered. For each measurement was calculated the total DM of the different structure (Leaves, stem and roots) of the tree considering the equations (4), (5), (6), (7), (8) and (9) presented in Chapter II. DM difference between measurements determined the tree growth during this period, a total of three periods of growth were considered. Once determined the DM of the trees, this was related to the TN content, considering the equations (1) and (2) for CCI versus TN content of leaves and the mean values of TN content of stem and roots, all of this determined in the Chapter II. Therefore the determination of the tree N retention is as follows:

$$\text{N retention} = (DM_{leaves} * \%\ TN) + (DM_{stems} * \%\ TN) + (DM_{roots} * \%TN) \qquad \text{(Eq. 1)}$$

In the case of prairie, the means of the samples collected for each treatment were considered as the total DM of the fescue. The TN content of prairie was calculated using the equation (3) presented in Chapter II. Considering the above equation were determined 5 levels of N concentration depending on colour and the MN associated to the colour (Table 7).

After estimating N retention of trees and prairie, these values were added to obtain the overall treatment retention.

Table 7. Total nitrogen (TN) concentration associated to Munsell number (MN) designated to each colour of the Munsell System Leaf Color (MSLC).

MN	TN
	-- % --
1	0.293
2	0.883
3	1.473
4	2.063
5	2.653

Statistical analysis

An analysis of variance (ANOVA) with *p*-value <0.05 was used to determine significant differences between the results of N retention in the treatments. The ANOVA considered the multiple comparison test of Tukey.

Based on the results obtained for each treatments in both sites, treatments N retentions values was compared by Student paired t-test, searching differences at significance level of *p*-value <0.05.

All the previous statistical analyses were performed using InfoStat statistical program.

RESULTS AND DISCUSSION

Growth and nitrogen retention of trees

Four measurements were carried out in trees in CLC: the first measurement was at initial state of the plants, and the other three measurements were used to estimate the growth of *L. chequen* and *D. winteri*. For both tree species there were found a positive growth in H and BD during the study period (11 months), except in the last measurement of *D. winteri* when the mean of H was minor than in the third measurement (see Table 8). The average H of *D. winteri* ranged from 50.5 cm in August 2013 to 68.2 cm in July 2014, whereas the H of *L. chequen* ranged from 98.7 to 118.8 cm. The BD of *D. winteri* ranged from 0.79 cm in the initial state to 1.28 cm in the last measurement, while *L. chequen* ranged from 0.94 cm to 1.50 cm. The difference between the initial state and the last measurement for both trees was about 20 cm in H and about 0.5 cm in the BD. In addition, *D. winteri* and *L. chequen* showed a good tolerance to the hypoxic conditions in CLC, especially during winter; while the fatalities were registered especially during summer when the soil had minor water availability and the hypoxic zone was lower. Tapia and Villavicencio (2007) using other native species, such as Maqui, Maitén, Chilco, Peumo, Coigüe, Quillay and Pimiento, found that these species showed a null adaptation to prolonged flooding periods in these soils.

Table 8. Growth parameters and chlorophyll content index (CCI) measurements for tree vegetal component of the buffer strips in the study site El Caleuche.

Measurement	Cumulative time	Treatment[1]	Parameters[2]		
			H	BD	CCI
	months		--------- cm	---------	-
0	0	GST1	98.7 ± 43.9	0.94 ± 0.47	81.5 ± 34.3
		GST2	50.5 ± 15.9	0.79 ± 0.18	56.0 ± 29.2
1	4	GST1	107.3 ± 45.9	1.24 ± 0.52	55.9 ± 15.6
		GST2	56.9 ± 21.1	1.08 ± 0.30	61.4 ± 21.7
2	7	GST1	116.4 ± 47.6	1.47 ± 0.58	59.4 ± 17.3
		GST2	69.8 ± 21.5	1.17 ± 0.32	46.8 ± 20.5
3	11	GST1	118.8 ± 51.2	1.50 ± 0.67	83.4 ± 21.0
		GST2	68.2 ± 21.1	1.28 ± 0.34	73.8 ± 30.8

[1] See Table 6. [2] H is height and BD is basal trunk diameter .

In contrast, the planting in SL was not possible and no measurements were carried out. Both tree species showed a bad adaptation to the conditions of SL, where the soil had a sandy loam surface textural class. The soil type was important for the adaptation of *D. winteri* and *L. chequen*, because both trees were associated with places nearby to water bodies or high water content in the soil, when even in the Mediterranean region can be found growing together (Abarzúa *et al.*, 2006). Therefore, is important to considered all possible variables to the selection of the vegetal components for the BS, such as its resistance to tolerate water or salt stress, the pollutant to control, the cost of plant material and even the interests of the farmer.

Table 9 shows the results of DM for trees in CLC, considering only BD to estimate the DM of *L. chequen* and *D. winteri*. The allometric relationships is shown in Chapter II are an integral part to estimate tree growth and therefore N uptake. The DM together with the CCI measurements was used to estimate the N retention for the tree vegetal component of the BS as is shown in Table 9.

Table 9. Total dry matter (DM) and nitrogen (N) uptake on the tree vegetal component of the buffer strips in the El Caleuche study site.

Measurement	Treatment[1]	DM	N uptake
		----------- kg ha^{-1} -----------	
1	GST1	22.8 ± 5.09	0.29 ± 0.08
	GST2	75.48 ± 53.48	2.88 ± 3.04
2	GST1	20.13 ± 5.29	0.26 ± 0.08
	GST2	19.59 ± 11.88	0.68 ± 0.77
3	GST1	8.11 ± 0.47	0.12 ± 0.00
	GST2	26.91 ± 6.41	1.46 ± 0.94
Total	GST1	51.04 ± 10.07	0.67 ± 0.16
	GST2	121.99 ± 58.44	5.01 ± 4.62

[1] See Table 6.

The mean values of DM that present *L. chequen* and *D. winteri* were 51 kg ha^{-1} and 122 kg ha^{-1}, respectively. Other studies showed higher values of DM production, for instance: Uri e*t al*. (2007) measured the DM in silver birch ranging from 6.0 to 22.9 ton ha^{-1} and Tufekcioglu *et al*. (2003) in poplar after seven years found aboveground biomass of 38 ton ha^{-1}. Fortier *et al*. (2015) in poplar shown aboveground biomass accumulation of 55 to 194 ton ha^{-1} after 9 growing seasons. Other studies reported an increase of 4.7 ton ha^{-1} of biomass in orange during a growing season considering only woody organs and leaves (Roccuzzo *et al*., 2012). Although, the DM production of *L. chequen* and *D. winteri* are lower than reported for other species, other studies consider a much longer period of evaluation, where the tree can show a better performance. Considering that one of the main reason for choosing a plant for BS is its capacity for growth the two Chilean native species would not be appropriate for BS, however, as already stated BS can provide another benefits where the use of native species could be important.

It is important to note that there is a lack of information about tree N uptake capacity in BS. For *L. chequen* and *D. winteri* the mean values of N uptake was 0.67 and 5.01 kg N ha^{-1}, respectively. These results were consistent with the N concentration in the different structure of the trees, where *D. winteri* presented higher N concentration. These results are lower than the reported by other authors, for instance in poplar Tufekcioglu *et al*. (2003)

found an N assimilation of 37 kg N ha^{-1}and Fortier e*t al*. (2015) in poplar reported a large variation of N uptake with values ranging from 277 kg N ha^{-1} to 872 kg N ha^{-1} after 9 growing seasons. Uri *et al*. (2007) in silver birch found N uptake ranging from 42.5 kg ha^{-1} to 168.8 kg ha^{-1}. Roccuzzo *et al*. (2012) reported for orange N uptake of 43 kg N ha^{-1} in a growing season and it was increased to 73 kg N ha^{-1} in the fruit. However, these results are from trees between 7 and 40 year-old growing in commercial orchard. Thus similar to the production of biomass, in this study the tree N uptake should consider a longer period of evaluation. Considering the results present in this study, *D. winteri* had the best capacity to N uptake and it should be a better option to be included in a BS. At the same time, the above results showed only absorption and storage of the tested plant species, so the data presented was just a part of the total N removal effect that provide a tree in a BS.

Growth and nitrogen retention of prairie

The establishment of the prairie for both sites was carried out during July and August 2012 and the first measurement was in October 2013 and November 2013 for CLC and SL, respectively; while the last cut was carried out in June and July 2014. The mean values of the DM production for the prairie ranged from 13.5 to 16.2 ton ha^{-1} in two season growing (Tables 10 and 11).

Table 10. Dry matter (DM) and nitrogen (N) uptake on the prairie component of the buffer strips in the study site El Caleuche.

Measurement	Treatment[1]	DM	N uptake
		-----kg ha^{-1}-----	
1	GST1	3.689 ± 326	74 ± 10
	GST2	4.249 ± 484	88 ± 10
	G	6.455 ± 2.195	149 ± 78
2	GST1	3.494 ± 1.476	51 ± 22
	GST2	6.872 ± 3.599	101 ± 53
	G	1.843 ± 19.39	28 ± 28
3	GST1	8.433 ± 5.165	168 ± 116
	GST2	4.040 ± 5.830	61 ± 84
	G	5.263 ± 2.598	109 ± 54
Total	GST1	15.616 ± 3.842	294 ± 85
	GST2	15.161 ± 2.266	250 ± 29
	G	13.561 ± 350	285 ± 44

[1] See Table 6.

These values are similar than the reported by Carter and Gregorich (2010) with a *F. arundinaceae* production between 2.6 to 12.7 ton DM ha^{-1} yr^{-1}. Sullivan *et al.* (1999) also reported maximum accumulative biomass for *F. arundinaceae* of 12.3 ton DM ha^{-1} yr^{-1}. Similarly, Ortega *et al.* (2013) reported *F. arundinaceae* production in South of Chile with total yield between 6.9 ton DM ha^{-1} yr^{-1} to 14.2 ton DM ha^{-1} yr^{-1}. Similarly Tapia and Villavicencio (2007) obtained DM mean values of 14.4 Mg ha^{-1} yr^{-1} in the commune of Pichidegua, where the prairie was a 30% of *F. arundinacea*.

The N uptake during the measurement period varied between 250 and 338 kg N ha^{-1} among treatments and study sites (Table 10 and Table 11). It have been reported values for fescue absorption ranging from 80 to 123 kg N ha^{-1} yr^{-1} (Ducnuigeen *et al.*, 1997; Sullivan *et al.*, 1999), similar to those determined in this study that considered two growing season of the prairie. Nuñez *et al.* (2010) on a permanent pasture incorporating *F. arundinacea* reported N uptake ranging from 175.7 to 303.7 kg N ha^{-1} yr^{-1}. Tapia and Villavicencio (2007) in the commune of Pichidegua obtained values between 248 and 457 kg N ha^{-1} yr^{-1} during a growing season of a combine prairie of *F. arundinacea* and *L. perenne*.

Table 11. Dry matter (DM) and nitrogen (N) uptake on the prairie component of the buffer strips in the study site San Luis.

Measurement	Treatment[1]	DM	N uptake
		-----kg ha^{-1}-----	
1	GST1	4.818 ± 1.845	68 ± 38
	GST2	6.231 ± 1.919	93 ± 28
	G	5.071 ± 75	75 ± 13
2	GST1	4.598 ± 1.840	95 ± 38
	GST2	6.138 ± 897	127 ± 19
	G	4.545 ± 108	108 ± 79
3	GST1	5.749 ± 3.142	112 ± 73
	GST2	1.934 ± 2.377	40 ± 49
	G	6.584 ± 156	156 ± 45
Total	GST1	15.165 ± 6.188	275 ± 133
	GST2	14.303 ± 3.371	260 ± 58
	G	16.200 ± 952	338 ± 47

[1] See Table 6.

Total nitrogen retention

Considering the above the TN retention it is presented en the Figure 7 and 8. In the case of SL TN retention did not include the contribution of the trees because these could not be measured. However, in CLC the contribution of the trees to the TN retention was low with values ranging from 0.001 to 0.025 kg $plot^{-1}$. Then the contribution in terms of removal of N by plants was mainly in N uptake by the prairie.

As was noted in Figure 7, it was found non statistically significant differences ($p > 0.05$) between treatments in CLC. However in SL there were found statistically significant differences between treatments (p<0.05), being the treatment G different from GST1 and GST2. Similarly for both sites the treatment G had the highest N uptake from the soil, where in this treatment the prairie covered a 100% of the surface.

Fortier *et al.* (2010) reported that the NO_3^- supply rate is the main factor controlling biomass growth and consequently nutrient accumulation in poplars, but this tree specie showed a large variation in N uptake. In both sites the N uptake presented a great variability for both prairie and trees (Table 9, 10 and 11), which could be associated with the high variability in the N content of the soil of the study sites (Rojas, 2015).

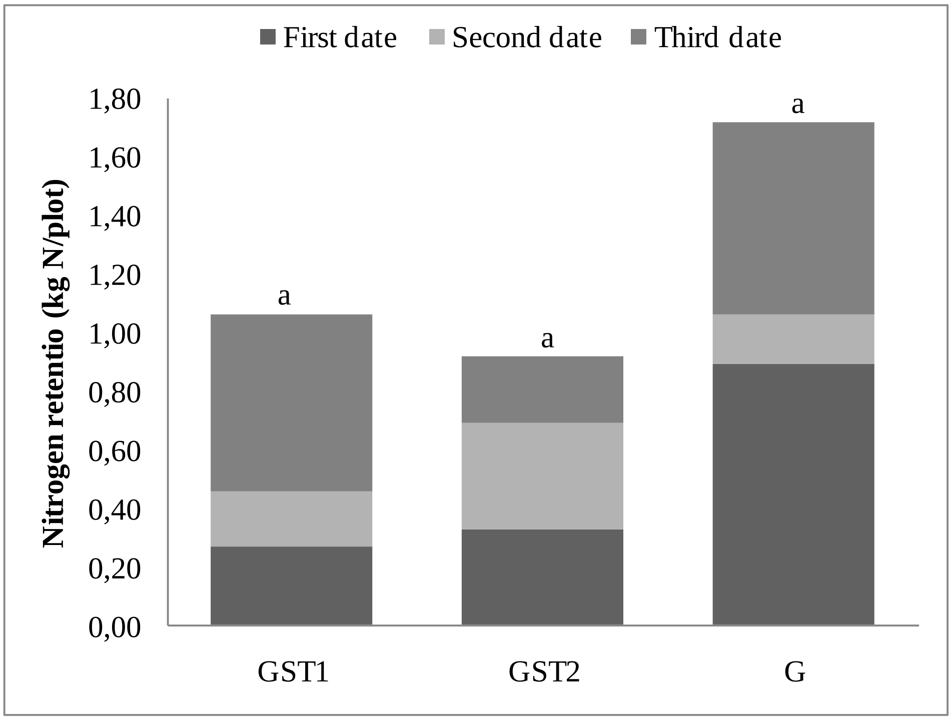

Figure 7. Nitrogen retention of the different treatments in El Caleuche study sites. Means with different letter are significantly different (p< 0.05, ANOVA).

Although there is a direct effect in the N retention of vegetation by the N uptake, it is necessary to consider the N incorporation in the litter production of vegetation and also the indirect role on N removal by stimulation denitrification activity through the supply of organic matter to the system. The latter is considered the most dominant and important process in the N removal in riparian sites, especially because the temporary character of the N uptake by the plant (Hefting *et al.*, 2005). Therefore, the denitrification process would be more important in sites such as CLC due to the permanent hypoxic soil condition.

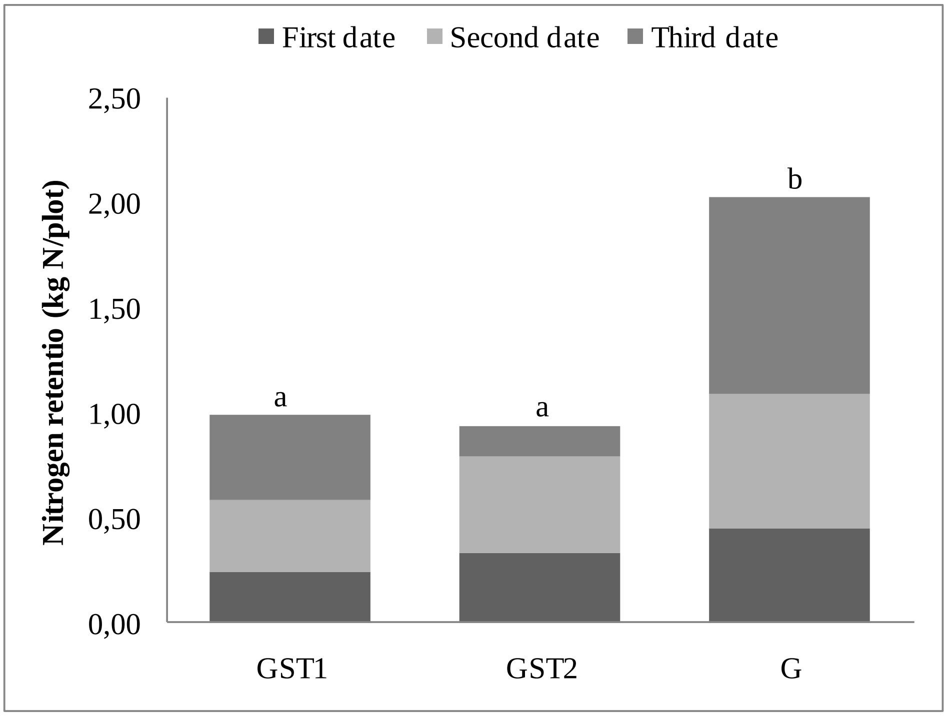

Figure 8. Nitrogen retention of the different treatments in San Luis study sites. Means with different letter are significantly different (*p*< 0.05, ANOVA).

Comparison between sites

The comparison between sites did not shown significantly differences (*p*>0.05, student-test) in any treatment (Table 12). Although between the sites did not exist significantly differences, it is important to emphasize that in SL sites most of the *D. winteri* and *L. chequen* trees could not be set due to the natural conditions in the site with a low water availability because of its coarse texture. Therefore in a long-term study (> 5 years) the effect of the trees in the CLC site could be more important.

Table 12. Comparison of nitrogen (N) retention in GST1 , GST2 and G treatments San Luis sites and Caleuche. Means with different letter in a column are significantly different (*p*< 0.05, ANOVA).

Treatment[1]	Caleuche	San Luis
	-----kg N plot^{-1}-----	
GST1	1.06 ± 0.31 a	0.99 ± 0.48 a
GST2	0.90 ± 0.10 a	0.93 ± 0.21 a
G	1.71 ± 0.26 a	2.03 ± 0.28 a

[1] See Table 6.

CONCLUSIONS

The application of calibrated non-destructives techniques showed to be useful for estimation of the N retention in the vegetation components of buffers strips (BS). The shrubs and trees used were too small to have a detectable impact on N uptake, therefore, N uptake was mainly attributable to the grass component. Thus the N retention comparison among treatments showed that grass was effective in N retention capacity of the BS, whereas young trees showed limited N retention in the same period. The effect of the tree component need to be evaluated in a long-time study period.

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APPENDIX I

Different colours identified in samples of *F. Arundinacea*.

Colour	Munsell Number	N (%)
5GY 6/8	2	0.883
5GY 6/6	3	1.473
5GY 5/8	3	1.473
5GY 5/6	3	1.473
5GY 4/8	3	1.473
5GY 4/6	4	2.063
5GY 4/4	4	2.063
5GY 3/4	5	2.653
2,5GY 6/8	2	0.883
2,5GY 6/6	3	1.473
2,5GY 5/8	2	0.883
2,5GY 5/6	3	1.473