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INTERACCIONES ECOSISTÉMICAS Y CAMBIOS EN LAS RELACIONES PRATENSES AL MEJORAR PRADERAS NATURALIZADAS DEGRADADAS CON PASTOREO OVINO INTENSIVO

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INTERACCIONES ECOSISTÉMICAS Y CAMBIOS EN LAS RELACIONES PRATENSES AL MEJORAR PRADERAS NATURALIZADAS DEGRADADAS CON PASTOREO OVINO INTENSIVO

Tesis presentada a la Facultad de Ciencias Agrarias de la Universidad Austral de Chile en cumplimiento parcial de los requisitos para optar al grado de Doctor en Ciencias Agrarias

Por

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Indice de abreviaciones

FP	Pradera naturalizada fertilizada
MS	Materia seca
NFP	Pradera naturalizada degradada

List of Abbreviations

Ac	*Agrostis capillaris* L.
Acm	*Agrostis capillaris* L. in mixture ring
ADF	Acid detergent fiber
AIC	Akaike Information Criterion
ASE	Average squared error
AI	Apparent intake
Au	Autumn
BLW	Total of flatweeds
Bv	*Bromus valdivianus* L.
CCI	Chlorophyll Content Index
CP	Crude protein
DM	Dry matter
DOMD	Digestible organic matter in the dry matter
FP	Fertilised pasture
HCG	High condition grass
HSh	Heavy shade
LCG	Low condition grass
Legs	Total of legumes
Llpre	Pre grazing lamina length
LLpost	Post grazing lamina length
Ln	*Leontodon nudicaulis* L.
Lp	*Lolium perenne* L.
Lpm	*Lolium perenne* L. in mixture ring
LWO	Lamina weight offered
ME	Metabolic energy
MSh	Medium shade
NFP	Non fertilised pasture

NDF	Neutral detergent fiber
OS	Open sun light
PAR	Photosynthetic active radiation
RMSD	Root means square deviations
Sp	Spring
Su	Summer
Wi	Winter
WSC	Water soluble carbohydrates

RESUMEN

Los estudios descritos en esta tesis evaluaron el mejoramiento de una pradera naturalizada degradada, a través de la fertilización y del pastoreo intensivo ovino controlado, evaluando aspectos de productividad, composición botánica, competencia de especies y selección animal.

El sitio de experimentación fue común en todos los ensayos, consistente en cuatro tratamientos: pradera naturalizada degradada (NFP), pradera naturalizada fertilizada (FP), pradera Mixta compuesta por *Lolium perenne* y *Trifolium repens* y pradera Diversa compuesta por *L. perenne*, *T. repens*, *Dactylis glomerata* y *Bromus valdivianus*. Todas las praderas fueron sometidas a pastoreo ovino intensivo. Todos los tratamientos fueron pastoreados por 25 ovejas.

El capítulo 1 describe el incremento de la producción y cambio en la composición botánica de praderas naturales degradadas luego de tres años de aplicación de fertilizantes y de pastoreo ovino intensivo. Las praderas Mixtas, Diversa y FP recibieron fertilización durante tres años de experimentación. En el transcurso del segundo de año de evaluación se evaluó la selectividad animal en *L. perenne*, *B. valdivianus* y *Leontodon nudicaulis*, especies características tanto en praderas permanentes que fueron sembradas, como en praderas naturalizadas en buena condición. FP alcanzó, al tercer año, una producción de materia seca, similar al de praderas sembradas ($P \geq 0.05$), junto con el incremento de especies de alta calidad forrajera. Además incrementó el nivel de nutrientes del suelo, disminuyendo el porcentaje de saturación de aluminio e incremento del pH. La selección de especies ejercida por los animales en pastoreo estuvo relacionada con la disponibilidad de matera seca de la pradera y con el largo de lámina. Así, cuando la productividad de la pradera incrementa (otoño y primavera) la probabilidad de pastoreo de especies *L. perenne* y *L. nudicaulis* aumenta en praderas que recibieron fertilización. Cuando la productividad de la pradera es restringida por la

disponibilidad de nutrientes del suelo o por estrés hídrico, la probabilidad de pastoreo aumenta en *L. nudicaulis* quien además presenta mayor largos de láminas.

El capítulo 2 tuvo como objetivo evaluar el efecto de la aplicación de fertilizantes en la dinámica de la población de macollos y competencia entre *L. perenne* y *Agrostis capillaris* en una pradera naturalizada degradada con y sin la aplicación de fertilizantes (NFP y FP). Para determinar la dinámica de macollamiento y competencia, se instalaron seis argollas de metal, las cuales contenían: 100% de macollos de *A. capillaris* (Ac), 100 % de macollos de *L. perenne* (Lp) o la mezcla de ambas especies en una proporción de 50% de macollos de *A. capillaris* – 50% de macollos de *L. perenne* (Acm-Lpm). La dinámica de la población de macollos fue registrada cada 15 días. *L. perenne* incrementó en la composición botánica de FP, en respuesta a la aplicación de fertilizantes, aumentando con ello la producción de materia seca total. La pradera naturalizada fertilizada obtuvo una densidad de macollos superior al de NFP con una densidad en la población de macollos de Lp/Lpm superior a Ac/Acm ($P \leq 0.05$).

El capítulo 3 evaluó la competencia por la luz como una variable restrictiva junto con la aplicación de fertilizantes en praderas naturalizadas degradadas. Se evaluó la densidad de macollos, largo de lámina, proteína cruda (CP), área foliar y el índice de concentración de clorofila (CCI) en *L. perenne* y *A. capillaris*. Se instauraron tres regímenes de luz incidente medidos como radiación fotosintéticamente activa (PAR): 60.7, 140.5 y 199.9 μmol $m^{-2}s^{-1}$ y medidos bajo estructuras cubiertas por malla raschel que representaron: alta sombra (Heavy shade; HSh), sombra media (Medium shade; MSh) y abierto a la luz (Open sunlight; OS), respectivamente. En las praderas NFP y FP bajo la sombra de cada estructura se colocaron 2 argollas las cuales contenían: 100% de macollos de *A. capillaris* (Ac), 100 % de macollos de *L. perenne* (Lp) o la mezcla de ambas especies: 50% *A. capillaris-L. perenne* (Acm-Lpm). El contenido de CP, CCI, largo de lámina y área foliar incrementó en la medida que la intensidad de radiación se redujo con *L. perenne* sobre *A. capillaris* en FP. En FP, Lp y Lpm tuvieron una alta producción de macollos en MSh y HSh. En épocas de estrés hídrico como en verano, el macollamiento de Ac fue superior al de Lp en OS.

En el capítulo 4 se desarrolló un análisis de árboles de decisión para investigar y predecir la abundancia de cuatro grupos funcionales de especies: especies de alta respuesta a la fertilización (HFRG; high fertility response grasses), especies tolerantes a bajos niveles de fertilidad (LFRG; low fertility response grasses), especies leguminosas y de hoja ancha en praderas naturalizadas del sur de Chile. Para ello se utilizó la información de los cuatros tratamientos del ensayo. Las variables más influyente en la abundancia de especies fueron: para HFRG, el nitrógeno mineral del suelo; para LFRG y especies de hoja ancha, Olsen P; y para leguminosas, pH del suelo. Este modelo de árbol de decisión reveló las principales variables influenciando la abundancia de cada uno de los grupos funcionales de especies a través de predicciones que podrían ser utilizadas como herramientas para planificar el manejo de la pradera.

En el capítulo 5 se desarrolló un modelo de árbol de decisión para determinar las variables ambientales de manejo más influyentes en el rendimiento anual y estacional de la pradera. Para ello se utilizó la información de los cuatros tratamientos del ensayo. Los resultados mostraron que el nitrógeno mineral del suelo es la variable más influyente en la productividad anual y de primavera, mientras que el pH del suelo, las precipitaciones y la saturación de aluminio son las más influyentes para verano, otoño e invierno, respectivamente. El resultado entregado por los árboles de decisión permitiría la utilización de estas predicciones dentro del manejo de la fertilización de la pradera para incrementar la productividad.

Palabras claves: mejoramiento, fertilización, selección de especies, competencia.

ABSTRACT

The studies described in this thesis evaluated the improvement of naturalized fertilised pasture through fertilization and under grazing intensive. Pasture herbage mass, botanical composition, species competition and animal selection were evaluated.

The experimental site was common in all the experiments with four treatments: Natural degraded pasture (NFP), Fertilised pasture (FP), Sown mixture pasture composed by *Lolium perenne* and *Trifolium repens*, and Sown diverse pasture composed by *L. perenne*, *T. repens*, *Dactylis glomerata* y *Bromus valdivianus*. All treatments were grazed by 25 sheep.

Chapter 1 describes the increase on herbage mass and botanical composition on natural degraded pastures after three years of fertiliser and grazing applied. Mixture, Diverse and FP were fertilised. On the second year of the experiment, animal selection was evaluated over *Lolium perenne*, *Bromus valdivianus* and *Leontodon nudicaulis* in permanent pastures which were sown and in naturalised pastures of high condition. At third year, FP achieved a pasture productivity similar to sown pastures ($P \geq 0.05$) with de the increase of high quality forage species. The soil nutrients and soil pH increased while soil aluminum saturation decreased. The selection of species during grazing was related with pasture availability and lamina length. When pasture productivity increased (Autumn and Spring) grazing probability of *L. perenne* and *B. valdivianus* increased on fertilised pastures (FP, Mixture and Diverse). When pasture herbage mass was restricted by low soil nutrients and drought stress the grazing probability increased to *L. nudicaulis* which had longest lamina.

Chapter 2 aimed to evaluate the effect of fertiliser on tiller dynamics and competition between *L. perenne* and *Agrostis capillaris* in a natural degraded pastures with and without fertiliser applied (FP and NFP). To evaluate tillering dynamics and competition process six metal rings were placed in each pasture containing: 100% of *A. capillaris* tillers (Ac), 100 % of

L. perenne tillers (Lp) or the mix of both species as 50% of *A. capillaris* – 50% of *L. perenne* tillers (Acm-Lpm). Tillering dynamics were counted every 15 days. *L. perenne* showed an increase in response to fertilisation in the botanical composition and pasture productivity. The naturalised fertilised pasture achieved a tiller density highest than NFP with a tiller population of Lp/Lpm over than Ac/Acm ($P \leq 0.05$).

Chapter 3 evaluated the light competition as a restrictive variable and the fertilization in natural degraded pastures. Tiller density, lamina length, crude protein (CP), foliar area and chlorophyll concentration index (CCI) were evaluated in *L. perenne* and *A. capillaris*. There were three incident light regimes measured as photosynthetic active radiation (PAR): 60.7, 140.5 and 199.9 μmol $m^{-2}s^{-1}$ and measured through shade structures covers with mesh which represented Heavy shade (HSh), Medium shade (MSh) and Open sunlight (OS), respectively. Each shade structure were placed in natural degraded pastures (NFP) and naturalised fertilised pastures (FP). Tiller dynamics were measured in six metal rings placed in the pastures under each structure. The rings (2 rings for each class) contained 100% *A. capillaris* tillers (Ac), 100 % *L. perenne* tillers (Lp) or mixture of both species: 50% *A. capillaris*-*L. perenne* (Acm-Lpm). The crude protein content, CCI, lamina length and leaf area increased as the shade increased with highest value recorded for FP in HSh with *L. perenne* over *A. capillaris*. In drought period as in summer, Ac tillering was higer than Lp in OS.

The study described in the chapter 4 a decision tree analyses were developed to investigate and predict the abundance of four functional groups of species as follow: high fertility responsive grasses (HFRG), low fertility tolerant grasses (LFRG), legumes and flatweeds in naturalised degraded pasture in southern of Chile. There were used the information registered of the four treatments. The most important variables influencing the species abundances were in HFRG, soil mineral nitrogen; in LFRG and flatweeds, Olsen P and for legumes, soil pH. The most important variables influencing the species abundances in HFRG was soil mineral nitrogen; in LFTG and flatweeds was Olsen P and for legumes was soil pH. The decision tree model revealed the most important variables influencing the

abundance of each functional group of species using predictions which could be used as tool to pasture management.

On the chapter 5 were developed decision tree analyses to determinate the most important variables influencing the annual and seasonal pasture productivity. There were used the information registered of the four treatments. The result showed that soil mineral nitrogen was the most important variable influencing the annual and spring productivity, while soil pH, rain and soil aluminium saturation were the most important variables for summer, autumn and winter, respectively. The result given by the models could be taken in to account to increase pasture production and to be included on fertiliser management strategies.

Key words: improvement, fertilization, species selection, competition.

INTRODUCCIÓN GENERAL

ANTECEDENTES GENERALES DE LAS PRADERAS DEL SUR DE CHILE

Las praderas del sur de Chile están compuestas por distintas comunidades de plantas cuya distribución varía de acuerdo a la disponibilidad de nutrientes del suelo. Estas praderas abarcan una superficie de 1.177.294,5 ha entre la décima y decimocuarta regiones, distribuidas en praderas naturalizadas (43.8%), praderas artificiales (8.5%) y praderas mejoradas (47.7%) (INE, 2007). La pradera naturalizada ha sido definida como una pradera compuesta por especies nativas e introducidas que crecen en conjunto (Butler et al., 1997). Su composición botánica es dominada principalmente por especies gramíneas (*Agrostis capillaris* L., *Holcus lanatus* L., *Lolium perenne* L., *Dactylis glomerata* L., *Bromus valdivianus* Phil., *Arrhenatherum elatius* spp bulbosum L., *Paspalum dasypleurum* Kunze, *Anthoxanthum odoratum* L. y *Lolium multiflorum* Lam.) y en menor proporción se encuentran especies de hoja ancha y leguminosas (<5 %) (Balocchi y López, 1996).

Las praderas naturalizadas son utilizadas para pastoreo sin recibir ningún tipo de fertilización que compense la extracción de nutrientes realizada por los animales, reflejando el mal manejo practicado durante muchos años. Por esta razón, presentan distintos grados de degradación del suelo, con una producción de materia seca entre 3 y 5 ton MS ha^{-1} $año^{-1}$ (Balocchi y López, 2009). El suelo donde se desarrollan las praderas naturalizadas es tipo Andisol de origen volcánico (Dörner et al., 2013). El principal problema que enfrentan los suelos de origen volcánico del sur de Chile, es la pérdida de la fertilidad en forma gradual y progresiva, situación que se ha producido, entre otros factores, por la alta susceptibilidad a la erosión, lo cual genera pérdida de la materia orgánica y de cationes del suelo (Campillo et al., 2005).

Esta susceptibilidad a la erosión, se ve incrementada por la alta pluviometría, por el uso intensivo del suelo en actividades agrícolas, por la introducción de especies de alto valor forrajero, por las fertilizaciones ineficientes, y en algunos casos, por el uso de fertilizantes de reacción ácida como urea, nitratos y fosfatos de amonio (White y Hodgson, 2000) que ayudan a incrementar la acidez de estos suelos. Se ha registrado pH ácido (<5.5), alto porcentaje de saturación de aluminio (>20%) (Mora et al., 2006), alta retención de fósforo, bajo nivel de potasio (68 $cmol+kg^{-1}$) y de bases intercambiables (potasio, sodio, calcio y magnesio 70.5 $cmol+kg^{-1}$) (Flores et al., 2013).

En la búsqueda de mejorar las prácticas de manejo de las praderas en el sur de Chile, se han utilizado dos estrategias para enfrentar este problema. Una de ellas es aumentar el nivel de nutrientes del suelo a través del establecimiento de cultivos (cultivos tradicionales, suplementarios y praderas de rotación corta) cuando la pradera está compuesta en un 100% de especies de bajo valor forrajero (*Agrostis capillaris*, *Poa annua*, *Leontodon nudicaulis*, *Taraxacum officinale*). Esto es por un periodo de tiempo (2 años) hasta lograr un nivel de fertilidad de suelo que permita la siembra de especies de alto valor forrajero como *Lolium perenne*, *Dactylis glomerata* y *Trifolium repens* (Torres, 2006). La siembra de las especies requiere de una preparación mecánica del suelo, por medio del arado y rastreado. Sin embargo, luego del transcurso de tres años, la pradera podría ser invadida por otras especies, por lo que su composición botánica es muy distinta a la de la siembra. Si la invasión de especies dentro de la pradera no disminuye la producción de materia seca, significa que la pradera aumentó su diversidad de especies (Sanderson et al., 2004).

Si la pradera degradada presenta un porcentaje de especies de medio y alto valor forrajero, es posible realizar una estrategia de recuperación de la pradera a través de procesos como la regulación de la carga animal, apotreramiento, el control del pastoreo, cortes de limpieza, control de plagas, el drenaje del suelo y la utilización de fertilizantes (Aydin y Uzun, 2005; Betteridge et al., 2011). Esto es relevante, ya que la implementación de un pastoreo controlado junto a la aplicación de fertilizantes, han obtenido producciones de materia seca cercanas a las 12 ton MS ha^{-1} $año^{-1}$ luego de tres años de aplicación de fertilizantes (nitrógeno, fósforo y potasio) (Campillo et al., 2005; Keim et al., 2014). Este manejo ha permitido, no sólo incrementar la producción (López y Valentine, 2003), sino que también mejorar la composición botánica de la pradera mediante la estimulación de los macollos de especies de alto valor forrajero (Lambert et al., 1983; McKenzie et al., 2006b; Shakhane et al., 2013).

La composición nutritiva de una pradera naturalizada fertilizada es similar al de praderas mixtas (*L. perenne/T. repens*) con altas concentraciones de proteína cruda (Keim et al., 2014) en relación a la aplicación de fertilizantes nitrogenados (Nordheim-Viken y Volden, 2009) y además tienen alta digestibilidad debido al incremento de especies de alta digestibilidad como *L. perenne* (Khaled et al., 2006). El aumento en la diversidad de especies establece un aumento de la estabilidad de la pradera frente a procesos estresantes del ambiente como el pastoreo y los periodos de sequía (McKenzie et al., 2006a; López et al., 2013). La aplicación de enmienda calcárea, junto con fertilizante han logrado incrementar el pH del suelo de 4.8 a 5.7 y el valor de Olsen P de 4.5 a 9.3 mg kg^{-1}, incrementando el rendimiento de la pradera (Poozech et al., 2010).

La estacionalidad en la producción es una de las características más importantes en el clima templado del sur de Chile, en donde se registran altas producciones durante el otoño y la primavera. Los más bajos rendimientos están asociados a las bajas temperaturas invernales y el estrés hídrico del verano (López et al., 2013). La restricción de agua del verano puede afectar negativamente número de especies como *L. perenne* mientras que especies tolerantes a este tipo de estrés como *B. valdivianus*, pueden dominar la pradera (Balocchi et al., 2001; Siebald, 2001). La diferencia entre ambas especies radica en que *B. valdivianus* tiene un mayor desarrollo de raíces, lo que le permite acceder al perfil profundo del suelo para captar agua (López et al., 2013). El contenido de agua del perfil de suelo puede disminuir en forma sostenida durante el verano hasta los 40 cm de profundidad (Dörner et al., 2010) y sólo el 20% del total del contenido de agua permanecer en los 5 cm (Dörner et al., 2012), profundidad en la cual especies de arraigamiento superficial como *L. perenne*, captan agua (Wedderburn et al., 2011).

Hace algunos años, Paladines y Muñoz (1982), señalaron que en Chile un importante número de praderas responden positivamente a la fertilización. La adición de nitrógeno, fósforo y otros nutrientes, junto con la corrección de la acidez del suelo incrementaron significativamente el rendimiento de la pradera. Por ejemplo, dosis de 120 kg N $ha^{-1}año^{-1}$, 120 kg P_2O_5 $ha^{-1}año^{-1}$, 100 kg K_2O $ha^{-1}año^{-1}$, 180 kg MgO $ha^{-1}año^{-1}$ y de 2000 kg ha^{-1} $año^{-1}$ de $CaCO_3$ sobre praderas naturalizadas obtuvieron 4200 kg MS ha^{-1} $año^{-1}$ más que una pradera degradada (Keim et al., 2014). Se ha determinado la positiva respuesta de la aplicación de fertilizante en otoño debido al aumento de la humedad luego de un verano seco permitiendo la mineralización del nitrógeno disponible en el fertilizante para las plantas (White y Hodgson, 2000). En primavera, también es recomendable la aplicación de fertilizantes, ya podrían existir deficiencias de nitrógeno producto de la lixiviación del nutriente por exceso de precipitaciones del invierno y por la disminución de especies leguminosas producto de las bajas temperaturas (White y Hodgson, 2000).

La utilización de nitrógeno como fertilizante ha sido muy popular en el mundo (Pennings et al., 2005, Megda y Monteiro, 2010) debido a que se le atribuye el incremento de la producción de materia seca de cultivos cuando se aplica durante el período de crecimiento de la planta (Crews y People, 2004; Monaghan et al., 2005). Así mismo, la aplicación de fertilizantes fosforados incrementa la proporción de leguminosas (Peoples et al., 2009). Este contenido de leguminosas podría disminuir a causa de procesos de competencia con especies gramíneas. La utilización de nitrógeno ha logrado disminuir un 50% el contenido inicial de leguminosas en praderas (Aydin y Uzun, 2005; Honsová et al., 2007; Guillingham et al., 2008).

Aún no se ha logrado establecer cuál es la mejor estrategia para mejorar una pradera naturalizada degradada, pero sí está claro que lo que se busca es maximizar la productividad y la persistencia. Factores de suelo, clima y ambiente definirán el tipo de especie que se desarrollará en la cubierta vegetal a través de las múltiples interacciones que existen entre ellos. Dentro de las interacciones existen componentes que se encuentran inmersos dentro del medio ambiente y que igualmente tienen efecto directo en el crecimiento de las plantas. La competencia por nutrientes del suelo, la selección de especies y la cantidad de luz interceptada por la pradera han sido estudiadas en torno a las adaptaciones de las especies frente a la sequía (Wedderburn et al., 2011; López et al., 2013), patrón espacial de las especies (Turnbull et al., 2004; Oksanen et al., 2006); intensidad de defoliación (Sbrissia et al., 2010), eficiencia fotosintética en sombreamiento (Devkota et al., 2009), pastoreo (Zaller et al., 2006), entre otros. En Chile la investigación sobre praderas naturalizadas se ha limitado en su mayoría a la productividad y composición botánica, siendo muy pocas las investigaciones que han abordado temas que incluyan las interacciones. Por lo tanto, el estudio de las interacciones ecosistémicas en praderas naturalizadas bajo pastoreo podría ser una herramienta vital para entender los principios fundamentales del mejoramiento.

SELECCIÓN DE ESPECIES

Los animales en pastoreo tienen la capacidad de seleccionar su alimento, especialmente ovinos, por sobre caprinos y bovinos (Mokoboki et al., 2011). La selección del alimento está dirigida a cubrir los requerimientos nutricionales y de evitar sustancias tóxicas (Provenza, 1995). Durante la selección los animales eligen un lugar para pastorear dentro de límites territoriales y de diversidad de especies (Fraser et al., 2009), seleccionando entre las especies y partes de la planta (Stuth, 1991). En una pradera el forraje disponible no está distribuido uniformemente en el plano vertical y horizontal, lo que provoca un consumo diferenciado de las plantas y de sus partes, vía selección (Carrére et al., 2003; Romney y Gill, 2000). Este tipo de selección ha logrado incrementar la producción de la pradera al estimular el rebrote de especies gramíneas (Liu et al., 2012), mejorando el valor nutritivo (Thomas et al., 2010).

La selección del pastoreo puede ser determinada mediante el marcaje de macollos (Betteridge et al., 1994; Hodgkinson et al., 2010). López et al. (2003) estudiaron la selectividad ovina por especies distribuidas en tres tipos de pendientes: alta, media y baja. Encontraron que durante la primavera se produjo un aumento en la selección de *A. capillaris*, especialmente en áreas con baja pendiente y de *A. odoratum* en áreas de media pendiente en relación a *L. perenne*. Con ello, las tres especies demostraron similar probabilidad de ser pastoreadas. Durante este período las ovejas no discriminaron entre macollos con diferentes longitudes de lámina en zonas de baja pendiente, pero si influyó en áreas de media pendiente. Este trabajo, además soportó la hipótesis de que las ovejas pueden distinguir entre especies al pastoreo y que la selección de especies varía durante el año en relación a la tasa de acumulación de forraje.

Efecto del manejo del pastoreo sobre la selección de especies

El manejo del pastoreo puede tener un gran efecto en la composición de la pradera (Shakhane et al., 2013) especialmente cuando las bajas temperaturas disminuyen la tasa de crecimiento y los animales seleccionan aquellas especies más nutritivas (Cook et al., 1978). Durante el verano, cuando las altas temperaturas y la sequía del verano van en desmedro del desarrollo de las especies, especies de hoja ancha invaden la pradera, especialmente cuando existen bajos niveles de fertilidad del suelo (Sanderson et al., 2004). En momentos del año cuando no existen las condiciones para el desarrollo de las especies, el manejo del pastoreo puede ser utilizado como un mecanismo de control de una especie sobre otra (Zaller, 2006). Sin embargo, el desarrollo de las especies pratenses siempre será marcado por las condiciones estacionales favorables (temperatura y humedad) y la fase reproductiva de la planta (Dowling et al., 2005).

Por otra parte, el sobrepastoreo puede ir en desmedro de la composición botánica al incrementar el número de especies de hoja ancha y la exclusión de leguminosas (Kemp y Culvenor, 1994; Dowling et al., 2006). Esta condición se desarrolla cuando el pastoreo no ha sido controlado. Cuando las condiciones de desarrollo están dadas y el pastoreo es controlado, una alta intensidad en la frecuencia de pastoreo incrementa la tasa de macollamiento de especies como *L. perenne* en comparación a una baja intensidad de pastoreo, al mismo tiempo que disminuye la densidad de puntos de crecimiento de *T. repens* (McKenzie et al., 2006a). El pastoreo extensivo, produce un cambio gradual en la diversidad de especies mostrando un aumento en el número de leguminosas presentes sobre la pradera (Marriot el al., 2009).

Cambios de las especies debido al pastoreo

La defoliación produce varios cambios en la fisiología de las especies. Por ejemplo, el corte de las láminas reduce la concentración de fotosintatos almacenados en las raíces de la planta (Erice et al., 2006), además de incrementar la digestibilidad, los carbohidratos solubles y la energía metabolizable (Lee et al., 2007). En pastoreos intensivos la tasa de aparición de hojas es estimulada como proceso compensatorio después de la defoliación (Lemaire et al., 2009). El índice de área foliar es un importante componente de la pradera el cual se adapta frente a variaciones en la intensidad de luz que llega a la lámina producto del sombreamiento entre especies (Lemaire y Chapman, 1996). En sombreamiento, los carbohidratos de reserva en la base de la planta se reducen al disminuir la luz (Erice et al., 2006) y con la defoliación, el área fotosintética e intercepción de la luz podrían ser afectados (Peri et al., 2007; Sbrissia et al., 2010; Abraham et al., 2014).

CONCEPTO DE COMPETENCIA

La competencia entre las especies ha sido considerada como uno de los factores determinantes en la riqueza de especies de una pradera (Grime, 1973, 1974). Las asociaciones que existen entre plantas dan lugar a que ocurran interacciones entre ellas, las cuales pueden tener un efecto negativo, positivo o neutro. Esto es debido a la convivencia con plantas vecinas de la misma o diferente especie en un ambiente determinado (Freckleton et al., 2009). La convivencia, en general, causa un efecto en la tasa de crecimiento, debido a la influencia que ejerce la presencia de una o más plantas (Kabba et al., 2007). Algunas especies se verán afectadas negativamente, prevaleciendo una jerarquía entre las especies predominantes sobre otras (Radosevich et al., 2007).

La competencia entre plantas de la misma especie se denomina "competencia intraespecífica", y se basa en el concepto de que las plantas por ser de la misma especie, suelen tener requerimientos muy parecidos, es por esto que compiten por los espacios, por sobrevivir y reproducirse (Bolnick et al., 2011). La competencia entre plantas de diferentes especies se denomina "competencia interespecífica", en donde ambas especies quieren ocupar el espacio de la otra para crecer y normalmente sus requerimientos no son iguales, por lo que la competencia se ve más marcada (McKenzie et al., 1999; Fridley et al., 2007).

Competencia por los nutrientes

La competencia entre plantas por los recursos del ambiente (nutrientes, luz, agua, espacio) es un proceso que ocurre en forma natural dentro de un ecosistema, el cual puede resultar en el desplazamiento de una especie, o bien, en una sucesión de especies con el cambio en el tamaño de la población (Grace y Tilman, 2012). El rol de la disponibilidad de nutrientes dentro del proceso de competencia es poco entendido en relación a la competencia entre las especies con características de crecimiento contrastantes (Ba et al., 2006). Existen dos teorías que tratan de explicar cómo es el proceso de competencia. Una de ellas fue propuesta por Grime (2006), quien señaló que la competencia podría intensificarse con el incremento de la productividad de la pradera, y que aquellos sitios de baja productividad tendrían una baja intensidad de competencia. En contraste, la teoría propuesta por Tilman (1988) señaló que la intensidad de competencia no cambia con la productividad y que la competencia podría ser intensa en sitios de baja productividad.

Existe varios estudios que señalan que la abundancia de especies, productividad y biomasa son la consecuencia de factores ambientales y de competencia interespecífica (Cahill, 2002; Hooper y Dukes, 2004). La convivencia entre dos plantas en donde se produce una competencia interespecífica ha sido considerada por algunos autores como una relación de beneficio mostrando un mayor desarrollo cuando la especie está creciendo en mezcla con otra especie que cuando está creciendo en monocultivo (Ba et al., 2006). Existe una positiva interacción entre mezclas de leguminosas y gramíneas debido a la fijación de nitrógeno por parte de leguminosas con el incremento del nitrógeno disponible para las especies gramíneas (Van Ruijven y Berendse, 2005). En algunas investigaciones a partir de la positiva interacción entre especies gramíneas y leguminosas, la competencia sugiere un incremento en la eficiencia de utilización de los nutrientes en gramíneas cuando se encuentra en un ambiente rico en recursos (Van Ruijven y Berendse, 2005).

Competencia por la luz

La mayoría de los estudios en relación a la competencia por la luz han sido realizados entre especies pratenses y árboles en sistemas silvopastoriles (Eastham y Rose, 1988; Dodd et al., 2005; Peri et al., 2006; Devkota et al., 2009). En ellos, la producción de forraje disminuyó en la medida que la densidad de la corona de los árboles incrementaba. La sombra que afecta a una especie ha sido considerada como una de las principales presiones competitivas (Guevara-Escobar et al., 1997) debido al efecto negativo de la falta de luz en la base de los macollos (Sparkes et al., 2006).

Luz interceptada por la planta

La luz que es interceptada por las plantas proviene de la energía solar transmitida entre una longitud de onda a 400-700 nm, más conocida como la radiación fotosintéticamente activa (PAR) (McCree, 1981). Esta energía es transmitida por la mayoría de las hojas y absorbida en las partes más altas de la canopia (Amthor, 2010). La luz disponible bajo la canopia es estimada por la medida PAR interceptado por la planta. La medición de PAR tiene la dificultad de que la intensidad de la luz transmitida puede variar de acuerdo a diferencias en la canopia y a la disponibilidad de luz solar (Devkota et al., 2009). Así, la fracción PAR absorbida por las hojas fluctuará en un 80% y 90% de la luz que llega a la planta. La determinación del valor exacto dependerá de cantidad de clorofila por unidad de superficie (Long et al., 2006). La productividad de la pradera es en base a la conversión de la energía solar, CO_2, nutrientes del suelo y agua (Amthor, 2010). La influencia de la intensidad de luz en la tasa de crecimiento ha sido determinada modificando la cantidad de fotones de luz que llegan a la planta a través de sombreamiento artificial (Devkota et al., 2009).

En general, el macollamiento y la producción de materia seca disminuyen por la reducción de la intensidad de luz, mientras que el índice de área foliar y largo de láminas se incrementan (Peri et al., 2006; Abraham et al., 2014). La magnitud de la disminución de la tasa de crecimiento de la planta al reducir la intensidad de luz, es influenciada por la cantidad de nutrientes del suelo, temperatura, humedad (Ericksen et al., 1981), estación del año y edad de la planta. La principal respuesta encontrada al reducir la intensidad de luz es el incremento del área foliar (Peri et al., 2006; Peri et al., 2007; Abraham et al., 2014). También existen efectos sobre el valor nutritivo (Devkota et al., 2009). El efecto de la reducción de la intensidad de luz no siempre produce una disminución en la tasa de crecimiento, sino que también podría existir un cambio en el desarrollo de la planta como mecanismo compensatorio adaptativo (Mitchell et al., 1998). Por lo tanto, la intensidad de luz que llega a la pradera es uno de los factores más críticos de producción y persistencia de una pradera bajo sombreamiento (Devkota et al., 2009).

MODELAMIENTO DE LA PRODUCTIVIDAD DE UNA PRADERA

El modelamiento de los antecedentes recopilados en una pradera ha sido ampliamente usado en agronomía y ecología para predecir el rendimiento de la pradera de una forma práctica y fácil de utilizar (Stuth et al., 1993; Moir et al., 2000; Zhang et al., 2005). Existen dos enfoques para modelar la información de la pradera, uno de ellos es el modelo mecanístico el cual revela factores causales de la productividad (Foy et al., 1999; Moir et al., 2000) y el modelo empírico, el cual es una forma de regresión que simula la productividad e investiga las interrelaciones con el ambiente (Lambert et al., 1983). El modelo mecanístico es ampliamente utilizado debido a la fortaleza de trabajar con una gran base de datos siendo más aplicable que el modelo empírico (Rickert et al., 2000). Sin embargo, el modelo empírico tiene la ventaja de ser altamente predictivo (Rickert et al., 2000) y la mejor opción para evaluar el impacto del ambiente y la aplicación de fertilizante en la producción de la pradera (Zhang et al., 2005).

Datamining es un proceso para consultar y extraer información útil sobre patrones y tendencias de la información que antes era desconocida. Los modelos de árboles de decisión son un tipo de datamining el cual ha tenido una creciente utilización en modelamiento ambiental de fácil interpretación (Yang et al., 2003) y con gran precisión en las predicciones (Zhang et al., 2005; Corson et al., 2007; Duff et al., 2012; Wan et al., 2009).

Por lo tanto, considerando la información disponible en cuanto al manejo de praderas naturalizadas, selección de especies, competencia por nutrientes y luz, se puede plantear la siguiente hipótesis: *El mejoramiento de una pradera naturalizada degradada, a través de la fertilización y del pastoreo intensivo ovino controlado, modifica la comunidad vegetal, ocurriendo un incremento en el número de especies competidoras por nutrientes, luz y poseedoras de un alto valor forrajero y selectividad animal*. La puesta a prueba de estas variables podría generar herramientas que permitan incidir sobre las estrategias de mejoramiento de praderas naturalizadas, de manera de poder maximizar el potencial productivo y persistencia de la pradera, de acuerdo a la respuesta de las especies frente a la selección de ellas por los animales, fertilización y sombreamiento.

En forma complementaria se realizaron modelos de árboles de decisión para predecir la abundancia de grupos funcionales de especies y la productividad de la pradera (anual y estacional).

En síntesis, en base a los antecedentes descritos, y a la información presentada, se han propuesto las siguientes hipótesis y objetivos de trabajos:

HIPÓTESIS GLOBALES

i. La fertilización de una pradera naturalizada degradada sometida a pastoreo ovino intensivo, incrementa la producción y la disponibilidad de especies altamente tolerantes a la fertilización. Cuando la producción de la pradera incrementa, la oveja selecciona *Lolium perenne* L. y *Bromus valdivianus* L. por sobre *Leontodon nudicaulis* L. Sin embargo, cuando la productividad de la pradera disminuye, la oveja selecciona *Leontodon nudicaulis* L. por sobre ambas gramíneas.

ii. La fertilización de una pradera naturalizada degradada permite el establecimiento espontáneo y desarrollo de *Lolium perenne*, el cual por competencia desplaza a *Agrostis capillaris* de la pradera.

iii. La adición de fertilizante en una pradera naturalizada degradada y la reducción de la luz incidente permiten el desarrollo de *Lolium perenne* L. el cual por competencia desplaza a *Agrostis capillaris* L. incremetando su macollamiento y área foliar.

OBJETIVOS ESPECÍFICOS

a. Evaluar la sustentabilidad en el largo plazo, la dinámica vegetacional y los niveles de fertilidad del suelo, al mejorar praderas naturalizadas degradadas bajo pastoreo intensivo ovino.

b. Determinar el grado de selectividad que tienen los ovinos en pastoreo sobre *L. nudicaulis* con respecto a *L. perenne* y *B. valdivianus* y la variación estacional de ésta.

c. Evaluar la competencia entre *L. perenne* y *A. capillaris* en praderas permanentes bajo como respuesta a la fertilización y cuando la luz es una variable restrictiva.

d. Realizar modelos de árboles de decisión para el rendimiento anual y estacional de praderas naturalizadas, y de los grupos funcionales de especies que las componen.

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Chapter 1

Relationships between pasture improvement and species selected by grazing sheep

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ABSTRACT

Pastures in the south of Chile are the main feeding for livestock. The most of them are naturalised pastures with serious problems in soil fertility and acidity. To alleviate this constraints, fertilisers were applied in naturalised degraded pasture to improve pasture productivity, botanical composition and nutrient contents. There were four treatments: non fertilised pasture (NFP), sown pastures: *Lolium perenne* and *Trifolium repens* (Mixture) and *L. perenne*, *T. repens*, *Dactylis glomerata* and *Bromus valdivianus* (Diverse), naturalised fertilised pastures (FP) during three years (March 2010-March 2013). In the second year (April 2011-March 2012), a selective grazing measurement were carried out using tillers/plant (*L. perenne* and *B. valdivianus* / *L. nudicaulis*) in all pastures treatments. *L. nudicaulis* was the dominant specie in FP and NFP, while *L. perenne* and *B. valdivianus* were dominant in Mixture and Diverse. The results revealed that FP achieved similar pasture herbage mass, botanical composition and nutrient content to Mixture and Diverse. Soil nutrient contents showed that soil pH, organic matter and Olsen P increased in Mixture, Diverse and FP. Soil Al saturation decreased at third year of fertiliser and lime applied on FP. In FP, Mixture and Diverse, the grazing probability of *L. perenne* and *B. valdivianus* increased in Autumn and Spring, when both species had longer lamina, while in Summer, *L. nudicaulis* was the most selected specie.

Keywords: fertilization, pasture herbage mass, botanical composition, selection

1. INTRODUCTION

The pastures in southern of Chile are the main forage resource to livestock (Balocchi et al., 2002). There are about 1.3 million ha^{-1} of pastures (INE, 2007). A total of 48% of the naturalised pastures are under grazing, and do not receive any type of fertiliser to compensate soil nutrient deficiencies (INE, 2007). Therefore, these pastures present a degree of degradation, which is characterised by a low annual herbage mass production (less than 5 ton DM $ha^{-1}year^{-1}$) (Balocchi & López, 2007). To alleviate these constraints, strategies such as fertilization would increase pasture production (Kemp et al., 1996). In general, pasture improvement in south of Chile include the application of lime and fertilisers to improve pH deficiencies and herbage mass production (Keim et al., 2014). These combined managements have a positive effect on botanical composition (Tharmaraj et al., 2008) increasing species such as *Lolium perenne* L., *Bromus valdivianus* Phil. and *Holcus lanatus* L. achieving a dry matter (DM) production close to 14 t DM ha^{-1} $year^{-1}$ (Keim et al., 2014).

On the other hand, grazing animals have a great impact on pasture productivity (Baumont et al., 2005; Soder et al., 2007) because stimulate the tillering of perennial grasses and eliminate less tolerant species to grazing (Soder et al., 2007). Also, they stimulate the mobilization of photosynthate reserves, enhancing buds development, therefore tillering, and consequently increasing biomass (Liu et al., 2012). The animal can be selective between species and part of plants (Bullok & Marriot, 2000), increasing pasture quality (Baumont et al., 2005). The selective grazing generates a pasture structure, with areas intensively grazed and others less frequently grazed (Chapman et al., 2007; Rossignol et al., 2011). The intensive controlled grazing, provides a dense herbage mass and delaying the ability of flatweeds to invade (Zaller, 2006). Several studies focused in sheep grazing behaviour showed a pasture structure with a decreased in flatweeds while grasses and legume species increased (Hamilton et al., 1973; Black & Penny 1984; Newman et al., 1992; Evju et al., 2006; Villalba et al., 2010).

The sheep have the ability to select between leaf and stem, between plants within a pasture, discriminate between slope and species, and even may select species with higher nutritional value and digestibility (López et al., 2003; Fraser et al., 2009a). This grazing ability of sheep may stimulates individual plant regrowth (Liu et al., 2012), decreases the diversity of species (Fraser et al., 2009b) and increases the proportion of green leaf (Wallis de Vries & Daleboudt, 1994).

It has been shown that fertilisation and liming of a low production naturalised pasture, through spontaneous changes of the botanical composition, increases herbage production and nutritive value to similar values to that of a sown pasture (Keim et al., 2014). Selective grazing can highlight the succession of species in a pasture, stimulating the growth of some species more than others (Lopez et al., 2003). It has been reported contradictory results about sheep grazing selection. Some studies showed that sheep prefer to consume high quality species than the average of the available pasture (Stuth, 1991). In other cases sheep prefer forage with higher herbage mass than quality (Baumont et al., 2005).

The majority of these studies have been conducted under experimental conditions with one or two species, in vegetative or reproductive stage, and often in a short term. In more complex situations, like in natural ecosystems, predictions of diet selection or the impact on the species remains unclear. If such a selection does exist in sheep grazing naturalised pastures, it could provide valuable information about the relationship between pastures improvement and species selection.

From the available evidence, it was hypothesized that the fertilization of a naturalised degraded pasture under sheep intensive grazing, increases pasture production and the availability of species high fertility tolerant. When pasture production increases, sheep select *L. perenne* and *B. valdivianus* over *L. nudicaulis*. However, when pasture production decreases, sheep select *L. nudicaulis* over both grasses.

The objective of this study was to evaluate the pasture production, botanical composition and nutrient content on natural fertilised pastures over three years of improvement and to evaluate the selective defoliation of fast growing grass species and a stress tolerate flatweed species.

2. MATERIAL AND METHODS

2.1 Study area

The study was located at Santa Rosa Research Station of Universidad Austral de Chile (39º 47' 26'' S, 73º 14' 12'' W), Valdivia, Chile. The experimental site was situated at 25 m a.s.l., and 2350 mm of average annual rainfall. The soils are classified as Duric Hapludand Soils (IREN et al., 1978). The monthly rainfall and average temperature during the experimental period (October 2012-August 2013) are shown in Figure 1.

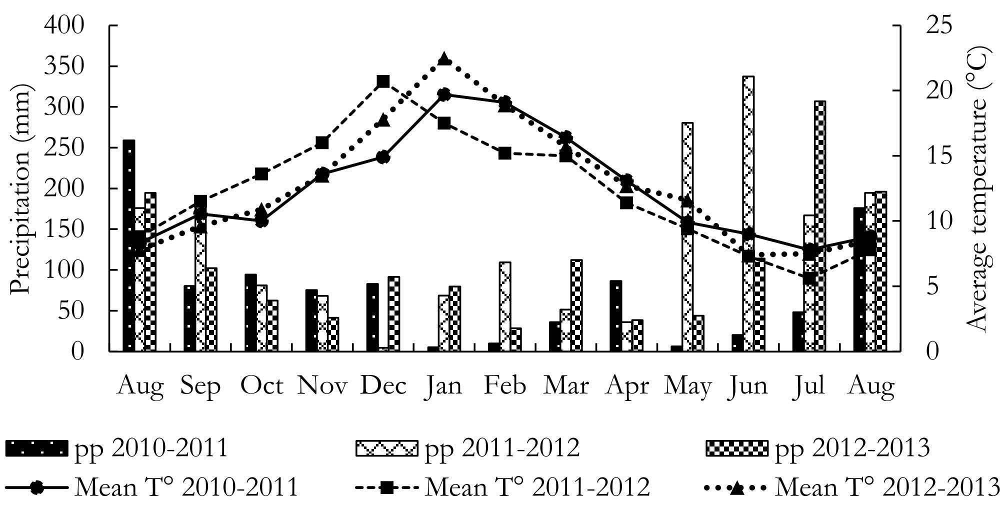

Figure 1. Monthly precipitation and mean temperature values during the experimental period in Valdivia

The initial soil water pH was 5.6, soil Olsen P 9.3 mg kg^{-1}, soil exchangeable potassium 68 mg kg^{-1} and 12.2% soil aluminium saturation. All chemical features of the soil were measured on the first 20 cm of the soil.

2.2 Treatments

The original pasture (naturalised non fertilised pasture: NFP) was found to be dominated by *Agrostis capillaris* L., *Anthoxanthum odoratum* L. and flatweeds. In April 2010, three methods of pasture improvement strategies were established: a) Naturalised fertilised and limed pasture (FP); b) Sown pasture (sowing rate: 25 kg ha^{-1} *Lolium perenne* L. cv. Arrow AR1 and 5 kg ha^{-1} *Trifolium repens* L. cv. Huia [Mixed]); c) Sown diverse pasture (sowing rate: 9 kg ha^{-1} *L. perenne* cv. Arrow AR1, 5 kg ha^{-1} *T. repens* cv. Huia, 4 kg ha^{-1} *Dactylis glomerata* L. cv. Ella and 27 kg ha^{-1} *Bromus valdivianus* Phil. nativo [Diverse]). Prior of the establishment of the improvements pastures (FP, Mixed and Diverse), the areas were sprayed with glyphosate using a dosage of 2.5 lt ha^{-1} of active ingredient. The Mixed and Diverse pastures were sown on March 2010.

NFP received no fertiliser application during the 3 years of evaluation. For FP, Mixed and Diverse, fertilisers and lime were applied annually to correct soil nutrients deficiencies (180 kg N ha^{-1} $year^{-1}$, 120 kg P ha^{-1} $year^{-1}$, 120 kg K ha^{-1} $year^{-1}$ and 2 t ha^{-1} $year^{-1}$ of $CaCO_3$ as lime). During the 3 years of the study, annually a rate of 45 kg N ha^{-1} of nitrogen fertiliser was applied in four opportunities. The other nutrients were applied once a year during April.

Each pasture plot (20 m x 20 m) was grazed by twenty five 2 years old sheep, Austral breed, live weight 55 ± 2.42 kg (average ± SEM) and body condition 2.71± 0.27 (average ± SEM) during the 3 years of the experiment. Each grazing event went for six hours. The three plots of each treatment were grazed at the same time when the pasture herbage mass reached an equivalent of 2.100 to 2.300 kg DM ha^{-1} until 1.000 to 1.200 kg DM ha^{-1} according to the pasture targets given by Matthews et al., (2004). The maximum grazing frequency was the 60 days, if the pasture did not reach the pregrazing herbage mass criteria, the plots were grazed (Hodgson et al., 2004).

2.3 Soil and pasture measurements

Daily temperature and soil water content were measured by sensors placed 10 cm deep in the soil. Soil fertility levels were determined once a year from April 2010 in each plot. In each plot, five soil samples were collected (0-20 cm soil, depth) in order to obtain a composed soil sample for each plot. The soil samples were air dried to determine pH, organic matter, soil mineral nitrogen, soil Olsen P and soil aluminium saturation (Sadzawka et al., 2006). Soil mineral nitrogen was measured as $N–NO_3$ and $N–NH_4$ using KCl as extract and Kjeldahl digestion method (Bremner, 1996). Soil Olsen P was determined by extraction with sodium bicarbonate (Olsen, 1954).

Pasture herbage mass was assessed using the rising plate method (Earle and McGowan 1979) with a calibrated equation: y=174,76x + 232,04 (R^2 0.8). The trim technique (Radcliffe et al., 1968) was used with a 0.1 m^2 quadrat and cutting the pasture to a soil level. Quality herbage was estimated in the treatments (Mixture, Diverse, FP and NFP) cutting the pasture above 4 cm to soil level in areas randomly chosen. The samples were dried at 60 °C for 48 in a forced air oven until constant weight and analysed for Crude protein (CP; Micro Kjeldahl method; AOAC, 1995), soluble protein (SP; Licitra et al., 1996), neutral detergent fibre (NDF; van Soest, 1967) and acid detergent fibre (ADF; AOAC, 1995) and digestible organic matter in the dry matter (DOMD, Tilley & Terry, 1963). Metabolizable energy (ME) was calculated from DOMD using the Garrido & Mann (1981) equation: ME (Mcal/kg DM) = 0.279 + 0.0325 × DOMD.

2.4 Prior selective grazing measurements

A preliminary study was conducted to determine a regression equation (linear, logistic and quadratic models) that better showed the relationship between lamina length and lamina dry matter weight (mg) for each of these three species: *L. perenne*, *B. valdivianus* and *L.nudicaulis* (L.) Banks ex Lowe. Thus, lamina herbage mass offered in pre-grazing and lamina dry matter consumption by the grazing animals were estimated based on the lamina length of the marked plants. The lamina mass consumption was obtained from the difference between pre and post-grazing lamina lengths. A total of 200 tillers/plants of *L. perenne*, *B. valdivianus* and *L. nudicaulis* in vegetative stage (lamina length range from 0.5 to18.2 cm, 0.2 to 17.3 cm and 9 to 19 cm, respectively) were collected (Figure 2).

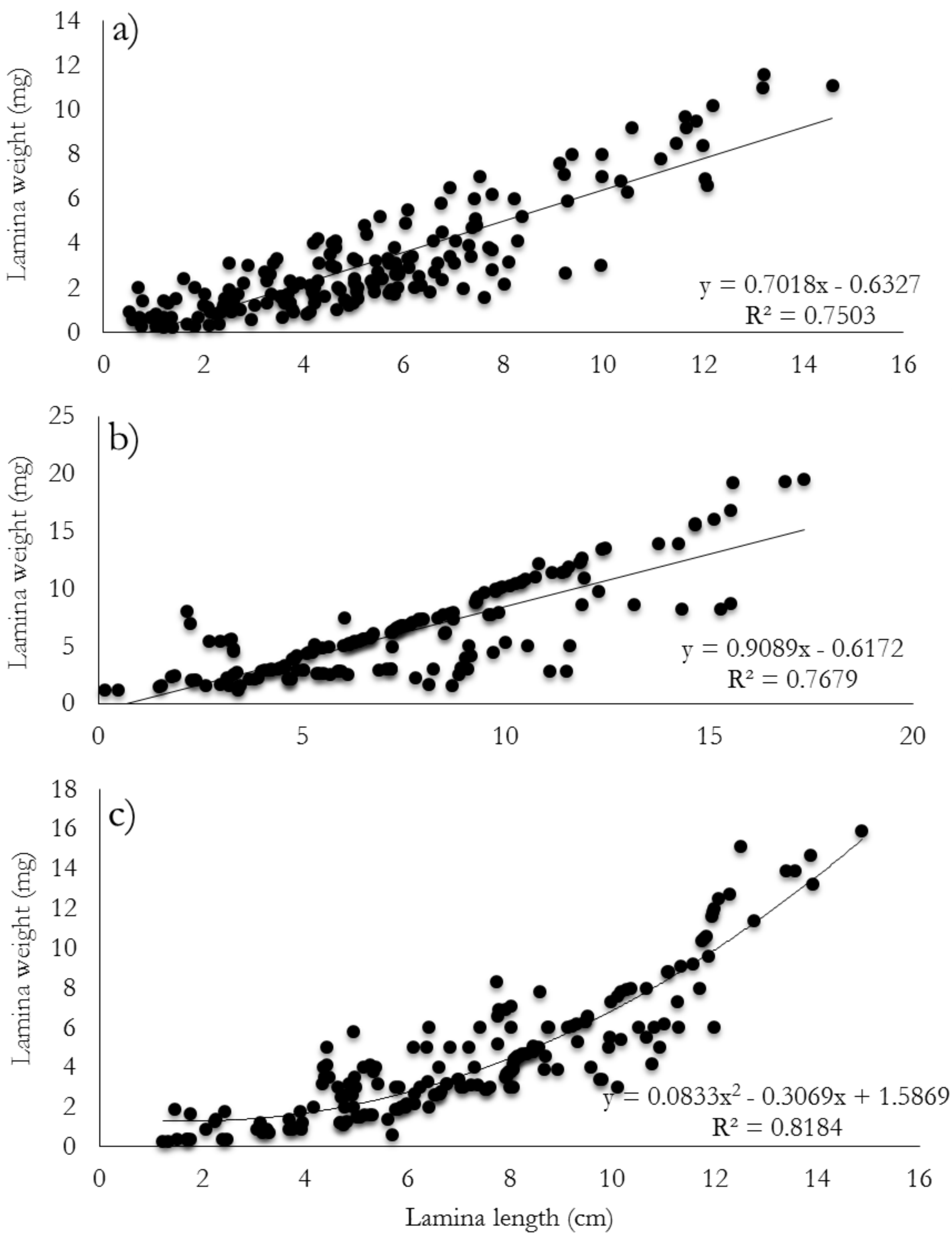

Figure 2. Models with the best fit to the data indicated the relationship between lamina length and lamina weigh (dry matter basis) for a) *L. perenne*, b) *B. valdivianus* and c) *L. nudicaulis*.

Each lamina was measured using calipers and dried in an oven at 60°C for 48 hrs. Subsequently leaf was weighed. Linear, logistic and quadratic models were fitted to the lamina weight and length for each species and separately using statistical program SAS, Version 12.3 (SAS Institute Inc, 2011) (Table 1). The Akaike Information Criterion (AIC) was determined for each equation. The equation that was appreciate have the best fit for each species was that with the lowest AIC (Fernandez, 2003).

2.5 Selective grazing measurement

The selective grazing evaluation was carried out during the second year since the pastures started being improved, from Autumn (April 2011) to end of Summer (March 2012). Selective grazing was achieved applying the technique reported by Hodgkinson et al., (2010). Three fixed transects of 15 m long were placed in each plot. Along each transect, every 0.75 m an individual tiller of *L. perenne* or *B. valdivianus* or a plant of *L. nudicaulis* was marked using a colored paperclip, which was attached to the soil by an eight cm nail. The marked tillers and plants were in a sequence following the same pattern along each transect. A total of 7 individual marked tillers or plants for each species were marked in each transect. In grasses, 3 leaves with a leaf stage between 0.75 and 0.25 were marked. In *L. nudicaulis*, 10 vegetative lamina were marked. Pre and post grazing, the nails were found using a metal detector allowing the identification of each tiller/plant marked. Plant height of each species was measured using a meter ruler. Each lamina length was measured from the tips of its ligules (*L. perenne* and *B. valdivianus*) or petiole base (*L. nudicaulis*). The tiller or plant that had a lamina that was shorter in post grazing was considered grazed, and a new tiller or plant was marked in a similar position. The transect was evaluated one each season during April (Autumn), July (Winter), October (Spring), January (Summer).

Crude protein (CP; Micro Kjeldahl method; AOAC, 1995), neutral detergent fibre (NDF; van Soest, 1967) and soluble carbohydrate concentration (WSC; Sabag, 1988) of *L.*

perenne, *B. valdivianus* and *L. nudicaulis* were determined. The samples were obtained before every grazing event, for each of these individual species, and cut at grazing level (4 cm height from soil level), dried at 60 °C for 48 hrs in a forced air oven until constant weight.

2.6 Statistical analyses

Treatments effects were analysed according to a randomized complete block design with factorial arrangement of the treatments with 4 type of pastures (Mixture, Diverse, FP and NFP) and 3 species within each type of pasture (*L. perenne*, *B. valdivianus* and *L. nudicaulis*). To determine statistical differences between treatments analysis of variance (ANOVA) was performed. To further analysis, LSD was applied to campare treatment means (Steel et al., 1997).

The statistical model was:

$$y_{ijkl} = \mu + \beta_i + \alpha_j + \tau_k + \alpha\tau_{jk} + \varepsilon_{ijkl}$$

Were:

y_{ijkl} = response pasture productivity and species selection evaluated variables between species

μ = the overall mean

β_i = block effect

α_j = four types of pastures

τ_k = selection effect

$\alpha\tau_{jk}$ = treatments interaction

ε_{ijkl} = random error

For each pasture species within each season (Autumn, Winter, Spring and Summer), the probability of the marked tillers/plants being grazed was determined in each pasture, by dividing the number of grazed tillers/plants, as shown by a shorter postgrazing lamina length compared to pregrazing lamina length, over the total number of the marked tillers/plants.

Each pasture species data were analysed separately using a generalised linear model with a binomial distribution (grazed=1; ungrazed=0) fitted using SAS PROC GENMOD (SAS, 2011), which indicated significant differences between the species within each season (Fernandez, 2003).

The amount of dry matter that was apparently consumed from each tiller was calculated using the lamina lengths (pre and post grazing) and the equation of best fit determined in the preliminary study. These values were compared between *L. perenne*, *B. valdivianus* and *L. nudicaulis* through ANOVA to determine whether the dry matter consumption differed between plant species within each season.

Principal component analysis (PCA) was performed to analyse the relationship between the nutritive value of the plant species (crude protein, water soluble carbohydrates and neutral detergent fibre) and morphological traits (pre and post grazing lamina length and lamina dry matter offered). All variables were standardized with mean zero and variance one prior to perform the PCA.

3. RESULTS

3.1 Pasture herbage mass, botanical composition and seasonal pasture growth

The pastures produced a significantly different amount of herbage mass a year (Table 1). Consistently during the whole evaluation NFP had significantly lower annual herbage mass production ($P \leq 0.001$) than the other pastures (Mixture, Diverse and FP). At the end of the third year the improved pastures, including FP, had a similar herbage mass production.

There were significant differences in botanical composition between pastures ($P \leq 0.001$) (Table 1). During the first year, Mixture and Diverse pastures had high contribution of *L. perenne* and *B. valdivianus*, while NFP and FP had a higher contribution of *L. nudicaulis*.

In the second year, *L. nudicaulis* increased its contribution in NFP, while in FP decreased, increasing *L. perenne*. In the third year, *L. nudicaulis* increased in all pastures, while *L. perenne* and *B. valdivianus* increased significantly in FP and decreased the low condition grasses contribution. Figure 3 illustrates monthly the daily average growth rates of the evaluated pastures across the three years. The highest growth rates were reached by the fertilised pastures (Mixture, Diverse and FP) with the growth peak in early October and March, while NFP reached the lowest growth rates. All the pastures had their lowest pasture growth rates during Summer and Winter.

Table 1. Pasture herbage mass production (kg DM $ha^{-1}y^{-1}$) and botanical composition (kg DM $ha^{-1}y^{-1}$) in three periods of evaluation.

Period	Pastures	Productivity	Ln	Lp	Bv	Hl	Tr	Ac	Legs	BLW	LCG	HCG
2010-2011	Mixture	9453^{a}	163^{d}	6521^{a}	1803^{a}	226^{c}	434^{c}	142^{d}	434^{b}	163^{d}	306^{c}	8550^{a}
	Diverse	7880^{c}	450^{c}	3148^{b}	1527^{a}	410^{b}	1289^{a}	391^{b}	1317^{a}	754^{c}	569^{b}	5241^{ab}
	NFP	4170^{d}	810^{b}	160^{d}	691^{b}	614^{a}	704^{b}	264^{c}	707^{b}	1369^{b}	378^{c}	3000^{c}
	FP	8415^{b}	2143^{a}	248^{c}	459^{c}	534^{b}	60^{d}	2865^{a}	60^{b}	3815^{a}	3212^{a}	1329^{d}
	sig	***	***	***	**	***	***	***	*	***	***	***
2011-2012	Mixture	10921^{a}	329^{c}	7065^{ab}	1355^{ab}	693^{c}	641^{a}	344^{b}	643^{a}	475^{c}	672^{b}	9131^{a}
	Diverse	10833^{a}	220^{c}	7936^{a}	1724^{a}	470^{cd}	233^{d}	97^{c}	233^{b}	297^{c}	149^{c}	10153^{a}
	NFP	5854^{c}	1259^{ab}	28^{d}	311^{c}	1044^{b}	593^{ab}	2013^{a}	621^{a}	1394^{b}	2375^{a}	1474^{c}
	FP	9443^{b}	1418^{a}	671^{c}	343^{c}	1878^{a}	444^{c}	2021^{a}	548^{a}	2202^{a}	2572^{a}	3123^{b}
	sig	***	**	**	**	***	***	***	*	**	**	***
2012-2013	Mixture	11550^{ab}	689^{c}	4731^{b}	1805^{b}	644^{a}	1343^{b}	1022^{b}	1350^{a}	1292^{c}	1289^{b}	7619^{b}
	Diverse	13734^{ab}	687^{c}	6281^{a}	2171^{a}	612^{a}	1719^{a}	823^{c}	1719^{a}	1379^{c}	1258^{b}	9378^{a}
	NFP	6188^{c}	1891^{b}	95^{d}	289^{d}	370^{b}	143^{d}	1766^{a}	215^{b}	3081^{b}	2055^{a}	836^{d}
	FP	12800^{a}	3151^{a}	1807^{c}	841^{c}	411^{b}	1130^{c}	1174^{b}	1302^{a}	5521^{a}	2286^{a}	3691^{c}
	sig	**	**	***	***	*	***	**	*	**	*	***

NFP, non fertilised pasture; FP, fertilised pasture; Ln, *Leontodon nudicaulis*, Lp, *Lolium perenne*; Bv, *Bromus valdivianus*; Hl, *Hocus lanatus*, Tr, *Trifolium repens*; Ac, *Agrostis capillaris*; Legs, total of legumes; BLW, total of flatweeds; LCG, low condition grass; HCG, high condition grass. *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; ns, not significant difference. Values on the same column followed by different letters present significant differences.

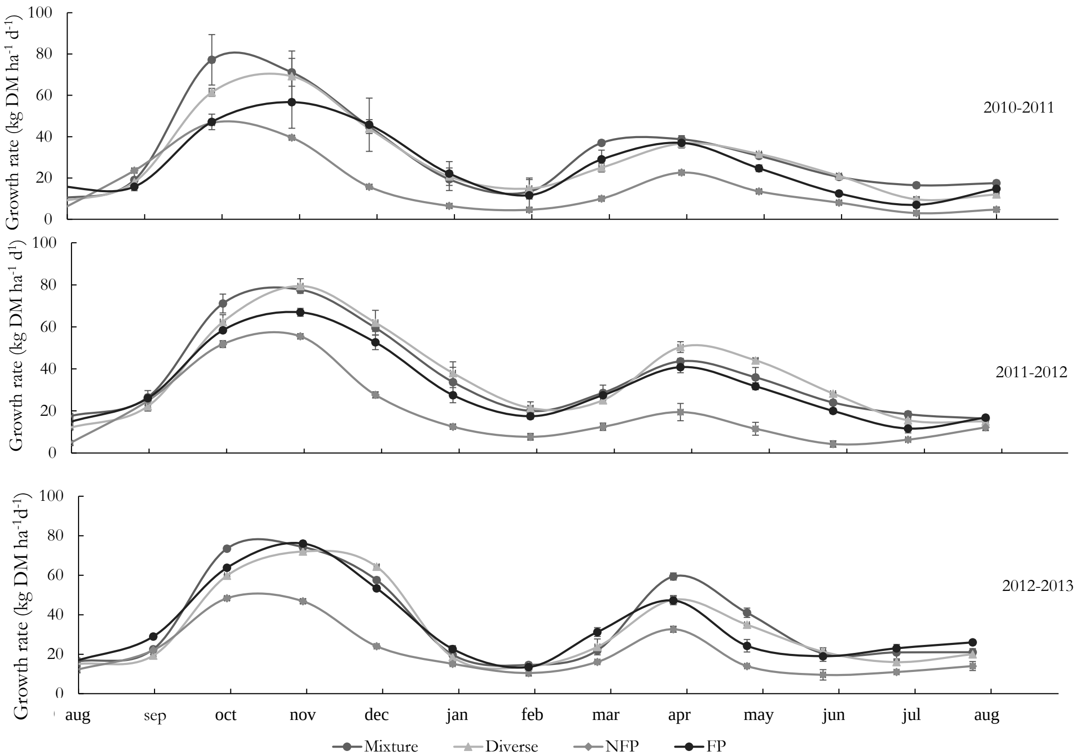

Figure 3. Monthly daily average growth rates (kg DM ha^{-1} d^{-1}) of pastures (Mixture, Diverse, non-fertilised pasture (NFP) and fertilised pasture (FP))

3.2 Seasonal nutritive value of pastures

The average of seasonal nutritive value (CP, SP, ME, WSC and NDF) of the pastures throughout the three years of evaluation are presented in Table 2. There were no statistical differences ($P \geq 0.05$) among the treatments for the CP content during Spring on first year. In Spring of the second year, FP had the highest CP content ($P \leq 0.05$) and NFP the lowest. No differences in CP content were observed during Autumn of third year ($P \geq 0.05$).

Statistical differences in the SP content of the pasture were found during all the experiment, thus the highest values were recorded in Autumn ($P \leq 0.01$) and Winter ($P \leq 0.01$).

There were significant differences among the treatments for the ME content, such that the highest values were recorded in Autumn and Winter (*P*≤0.05) in fertilised pastures.

The WSC were statistical different between pastures, such that the highest value was registered in Diverse pasture during Autumn (Table 2). FP showed the highest content in NDF during Autumn (*P*≤0.05) and Summer (*P*≤0.01) in the first year. In the second year, Diverse pasture showed the highest value during Autumn (*P*≤0.01). The highest value was in NFP during Spring (*P*≤0.05). On the third year, FP showed the highest NDF value (*P*≤0.01) in Autumn.

Table 2. Seasonal nutrient contents of the pastures throughout three years of evaluation.

	2011				2012				2013
	Au	Wi	Sp	Su	Au	Wi	Sp	Su	Au
Crude Protein (g kg^{-1} DM)									
Mixture	218^{ab}	265^{a}	248	210^{a}	226^{a}	230^{b}	246^{a}	187^{a}	241
Diverse	262^{a}	249^{b}	237	212^{a}	225^{a}	242^{a}	244^{a}	214^{a}	285
NFP	206^{b}	224^{c}	218	133^{b}	210^{b}	219^{b}	202^{b}	139^{b}	228
FP	261^{a}	251^{ab}	236	172^{ab}	229^{a}	229^{b}	253^{a}	205^{a}	284
sig	**	**	ns	*	*	*	*	*	ns
Soluble Protein (g kg^{-1} DM)									
Mixture	70b^{c}	96^{a}	91^{a}	81^{a}	108^{a}	115^{a}	87	88^{a}	116^{b}
Diverse	91a	91^{a}	83^{a}	93^{a}	103^{a}	138^{a}	86	81^{b}	139^{a}
NFP	66^{c}	70b	66^{b}	52^{b}	96^{a}	88^{b}	73	94^{a}	108^{c}
FP	88^{a}	86^{a}	88^{a}	78^{b}	85^{b}	92^{b}	77	79^{b}	141^{a}
sig	**	*	*	*	*	**	ns	*	**
Metabolizable Energy (MJ kg^{-1} DM)									
Mixture	10.7^{b}	11.5^{a}	10.7^{a}	9.7^{a}	10.4^{a}	10.7	10.5	11^{a}	11.8
Diverse	11.1^{a}	11.3^{a}	10.6^{a}	8.9^{ab}	10.4^{a}	10.5	10.6	10.1^{b}	11.1
NFP	10.3^{c}	10.5^{b}	9.5^{b}	8.3^{b}	9.2^{b}	10.5	10.1	10.5^{b}	11.3
FP	10.8^{b}	10.8^{ab}	10.5^{a}	8.7^{ab}	10.9^{a}	9.8	10.5	11.5^{b}	11.8
sig	**	**	*	*	*	ns	ns	*	ns
Water Soluble Carbohidrates (g kg^{-1} DM)									
Mixture	91^{b}	87^{ab}	69	74	85^{b}	81	60^{b}	92^{b}	70
Diverse	123^{a}	107^{a}	77	68	110^{a}	85	53^{b}	94^{b}	51
NFP	66^{c}	77^{b}	53	73	80^{b}	88	87^{a}	98.3^{a}	60
FP	87^{b}	93^{ab}	60	76	85^{b}	93	73^{b}	88^{b}	68
sig	**	*	ns	ns	*	ns	**	*	ns
Neutral Detergent Fibre (g kg^{-1} DM)									
Mixture	251^{b}	207^{b}	511	477^{b}	480^{a}	463	489^{b}	465^{ab}	371^{ab}
Diverse	273^{b}	251^{b}	479	534^{b}	501^{a}	416	493^{b}	482^{a}	329^{bc}
NFP	310^{a}	291^{a}	519	409^{c}	405^{b}	480	525^{a}	401^{b}	294^{c}
FP	325^{b}	263^{b}	517	545^{a}	491^{a}	473	486^{b}	457^{ab}	386^{a}
sig	*	**	ns	**	**	ns	*	**	**

NFP, non fertilised pastures; FP, fertilised pastures; Au, Autumn; Wi, Winter; Sp, Spring; Su, Summer. *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; ns, non significant difference. Values on the same column followed by different letters present significant differences.

3.3 Soil fertility status of the pastures

Soil nutrient status improved through the years (Table 4). Soil pH, OM and Olsen P increased in fertilised pastures (Mixture, Diverse and FP), while soil aluminium saturation significantly decreased by the third year of pasture improvement. Soil N mineral was higher in fertilised pasture than NFP, except at the beginning where Mixture pasture had the lower value for 2011.

Table 3. Soil nutrients of the pastures throughout three years of evaluation.

Period	Pastures	pH (1:2.5)	OM (%)	N mineral (mg kg^{-1})	Olsen P (mg kg^{-1})	Al saturation (%)
2011	Mixture	5.6	13.9	30.8	9.8	5.4^{b}
	Diverse	5.6	13.3	32.2	8.7	6.9^{b}
	NFP	5.5	14.4	31.3	9.7	13.9^{a}
	FP	5.7	13.8	41.1	10.1	5.9^{b}
	sig	ns	ns	ns	ns	**
2012	Mixture	5.7	14.7	39.8^{b}	11.6	3.4^{c}
	Diverse	5.7	15.2	42^{b}	11.5	4.2^{b}
	NFP	5.6	15.2	30.2^{b}	9.4	7.9^{a}
	FP	5.9	14.6	49^{a}	11.5	2.8^{d}
	sig	ns	ns	*	ns	***
2013	Mixture	6.1^{a}	14.5	44.2^{a}	11.6	3.1^{b}
	Diverse	6.0^{a}	14.9	35^{ab}	11.9	3.9^{b}
	NFP	5.6^{b}	16.7	31.1^{b}	11.6	8.0^{a}
	FP	6.1^{a}	15.4	41.1^{a}	12.5	3.1^{b}
	sig	*	ns	*	ns	*

NFP, non fertilised pasture; FP, fertilised pasture; OM, organic matter; N mineral, nitrogen mineral; Al saturation, aluminium saturation. *P≤0.05; **P≤0.01; ***P≤0.001; ns, non significant difference. Values on the same column followed by different letters present significant differences.

3.4 Selective grazing, apparent intake and grazing probability

Regarding the relationship between lamina length and lamina weight for *L. perenne*, *B. valdivianus* and *L. nudicaulis*, the equations that best fitted them and their regression coefficient are in Table 4. The lowest Akaike Information Criterion (AIC) for Lp and Bv were obtained with linear equations (0.7018x-0.6327 and 0.9087x-0.6172, respectively) and for Ln was through an quadratic equation ($0.0833x^2$-0.3069x+1.5859) (Figure 2).

Table 4. Model fitted to the relationship between lamina length for *L. perenne* (n: 200), *B. valdivianus* (n: 200) and *L. nudicaulis* (n: 200), regression coefficients (r^2) and Akaike Information Criterion (AIC).

Specie	Model fitted	r^2	AIC	sig
L. perenne	y: 0.7018x-0.6327	0.75	931.4	***
	y: 2.267ln(x)-0.3552	0.5	940.3	***
	y: 0.0453x2+0.1248x+0.733	0.7	932.1	***
B valdivianus	y: 0.9089x-0.6172	0.76	832.4	***
	y: 4.2068ln(x)-1.6737	0.41	844.3	***
	y: 0.0462x2+0.1404x+1.963	0.7	836.2	***
L. nudicaulis	y: 0.9323x-2.2634	0.74	1387.1	***
	y: 4.8164ln(x)4.5111	0.56	1380.3	***
	y: 0.0833x2-0.3069x+1.5859	0.81	1377.1	***

***$P \leq 0.001$

The probability of being grazed and the apparent intake of the mark tillers/plant were related to the type of pastures (Table 5). The grazing probability in Mixture, Diverse and FP was greater in *L. perenne* and in NFP, *L. nudicaulis* was the most grazed. In all pasture, Ln showe the greater intake by sheep (Table 5).

Table 5. Grazing probability and Apparent intake (mg, dry matter) per transect of the marked tillers of *L. perenne* and *B. valdivianus*, and plants of *L. nudicaulis* by the sheep in each pasture (mean ± sem) and statistical differences between species.

	Mixture	Diverse	NFP	FP
Grazing probability				
L. perenne	2.41±0.01[a]	2.52±0.03[a]	1.78±0.01[b]	2.32±0.01[a]
B. valdivianus	1.94±0.06[c]	2.05±0.05[b]	1.88±0.06[b]	2.26±0.01[a]
L. nudicaulis	2.11±0.03[b]	2±0.03[b]	2.51±0.05[a]	2.10±0.03[b]
sig	***	*	*	*
Apparent intake				
	Mixture	Diverse	NFP	FP
L. perenne	142± 3.8[b]	130± 12.1[b]	61.8± 8.1[b]	125± 7.9[b]
B. valdivianus	144± 9.5[b]	124± 11[c]	45.1± 8.4[c]	100± 7.1[c]
L. nudicaulis	481± 34.5[a]	278± 17.3[a]	386± 32.1[a]	284± 15.3[a]
sig	***	***	***	***

NF, non fertilised pasture; FP, fertilised pasture. *$P \leq 0.05$; $P \leq 0.01$; $P \leq 0.001$; ns, non significant difference.

Table 6. Grazing probability of tillers/plant from different type of pasture (Mixture, Diverse, non-fertilised pasture [NFP] and fertilised pasture [FP]) and species (*L. perenne*, *B. valdivianus* and *L. nudicaulis*) in each season.

	Autumn	Winter	Spring	Summer
Mixture	0.60 ± 0.03^{a}	0.51 ± 0.02^{ab}	0.65 ± 0.05^{a}	0.40 ± 0.03
Diverse	0.58 ± 0.02^{a}	0.53 ± 0.01^{ab}	0.60 ± 0.02^{a}	0.44 ± 0.01
NFP	0.45 ± 0.04^{b}	0.48 ± 0.01^{c}	0.51 ± 0.03^{b}	0.45 ± 0.01
FP	0.56 ± 0.02^{a}	0.58 ± 0.02^{a}	0.58 ± 0.02^{a}	0.43 ± 0.02
sig	*	**	*	ns
Lp	0.68 ± 0.05^{a}	0.56 ± 0.05^{a}	0.64 ± 0.01^{a}	0.56 ± 0.02^{a}
Bv	0.57 ± 0.03^{b}	0.55 ± 0.03^{a}	0.59 ± 0.02^{ab}	0.52 ± 0.03^{b}
Ln	0.60 ± 0.02^{b}	0.48 ± 0.09^{b}	0.56 ± 0.02^{b}	0.57 ± 0.01^{a}
sig	*	*	**	*
Mixture Lp	0.68 ± 0.05^{a}	0.49 ± 0.02^{b}	0.70 ± 0.06^{a}	0.54 ± 0.02^{a}
Mixture Bv	0.46 ± 0.06^{c}	0.56 ± 0.07^{a}	0.55 ± 0.02^{b}	0.37 ± 0.06^{b}
Mixture Ln	0.52 ± 0.02^{b}	0.46 ± 0.02^{b}	0.57 ± 0.03^{b}	0.56 ± 0.04^{a}
sig	***	*	*	**
Diverse Lp	0.71 ± 0.05^{a}	0.56 ± 0.03^{a}	0.63 ± 0.01^{b}	0.62 ± 0.01^{a}
Diverse Bv	0.52 ± 0.01^{b}	0.48 ± 0.01^{b}	0.65 ± 0.01^{a}	0.40 ± 0.01^{b}
Diverse Ln	0.46 ± 0.06^{c}	0.38 ± 0.03^{c}	0.56 ± 0.05^{c}	0.60 ± 0.04^{a}
sig	***	**	**	**
NFP Lp	0.48 ± 0.06^{c}	0.49 ± 0.06^{b}	0.41 ± 0.01^{b}	0.40 ± 0.03^{b}
NFP Bv	0.62 ± 0.03^{b}	0.48 ± 0.03^{b}	0.48 ± 0.03^{b}	0.30 ± 0.02^{c}
NFP Ln	0.70 ± 0.05^{a}	0.67 ± 0.02^{a}	0.60 ± 0.08^{a}	0.54 ± 0.01^{a}
sig	***	*	*	***
FP Lp	0.73 ± 0.02^{a}	0.52 ± 0.02^{b}	0.56 ± 0.04	0.51 ± 0.05^{a}
FP Bv	0.69 ± 0.09^{a}	0.62 ± 0.05^{a}	0.54 ± 0.06	0.41 ± 0.06^{b}
FP Ln	0.59 ± 0.09^{b}	0.51 ± 0.08^{b}	0.51 ± 0.07	0.49 ± 0.02^{b}
sig	*	*	ns	*

Lp, *L. perenne*; Bv, *B. valdivianus*; Ln, *L. nudicaulis* *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; ns, non significant difference. Values on the same column followed by the same letters present significant differences.

Table 7. Apparent intake consumed per transect of the marked tillers of *L. perenne* and *B. valdivianus*, and plants of *L. nudicaulis* by the sheep in each pasture per season (mean ± sem, mg dry matter) and statistical differences between species.

		Apparent intake			
	Season	Lp	Bv	Ln	sig
Mixture	Autumn	36.3 ± 0.61	37.03 ± 0.45	86.7 ± 4.3	**
	Winter	26.63 ± 1.6	22.1 ± 0.4	18 ± 1.1	*
	Spring	56.7 ± 3.98	53.6 ± 4.27	209.3 ± 22.2	***
	Summer	22.9 ± 0.3	31.5 ± 3.3	168 ± 32.2	***
Diverse	Autumn	36.8 ± 0.35	36.1 ± 1.13	48.6 ± 1.1	**
	Winter	27.8 ± 0.4	27.3 ± 0.8	33.3 ± 1.3	*
	Spring	43 ± 1.33	52.6 ± 2.08	128.2 ± 5.2	***
	Summer	22.8 ± 4.8	8.63 ± 0.8	68.6 ± 12.1	***
NFP	Autumn	17.8 ± 1.12	15.1 ± 1.26	53.2 ± 5.4	**
	Winter	6.48 ± 1.56	5.37 ± 0.93	45.9 ± 1.2	***
	Spring	19.4 ± 4.46	16.5 ± 0.13	165 ± 14.5	***
	Summer	18.2 ± 3.15	8.12 ± 1.53	122 ± 33.8	***
FP	Autumn	38.9 ± 0.25	37.8 ± 0.51	117 ± 5.65	***
	Winter	29.3 ± 1.15	26.5 ± 0.78	36.9 ± 1.8	*
	Spring	35.8 ± 3.18	26.4 ± 2.43	65 ± 8	**
	Summer	21.7 ± 2.98	9.71 ± 2.14	65.5 ± 7.7	**

NF, non fertilised pasture; FP, fertilised pasture; Lp, *Lolium perenne*; Bv, *Bromus valdivianus*; Ln, *Leontodon nudicaulis*. *$P \leq 0.05$; $P \leq 0.01$; $P \leq 0.001$; ns, non significant difference.

The probability of being grazed (Table 6) and the apparent intake (Table 7) of the mark tillers/plants were strongly related to the type of pasture and season. The apparently intake of Ln was significantly greater than Lp and Bv, but the probability of each species of being grazed were different between pastures. The differences between species respect to the grazing probability were not consistently. In the fertilised pastures, Lp and Bv were the more grazed during Autumn, Winter and Spring. On the other hand, Ln was the more consumed during

Summer. In NFP, Ln was selected over the other two species to be grazed across the four seasons.

Table 8. Lamina length (cm) of tillers/plant from different type of pasture (Mixture, Diverse, non-fertilised pasture [NFP] and fertilised pasture [FP]), species (*L. perenne*, *B. valdivianus* and *L. nudicaulis*) and defoliation selected (nonselected and selected).

	Mixture	Diverse	NFP	FP
L. perenne	11.1±0.9[b]	11.5±0.8[b]	7.1±0.3[b]	10.9±1.2[c]
B. valdivianus	12.4±1.1[a]	13.3±1.3[a]	8.5±0.9[b]	12.5±0.8[b]
L. nudicaulis	9.3±0.6[c]	10.9±1.1[c]	13.1±1.1[a]	13.7±0.9[a]
sig	***	***	**	**
Non selected	7.8±0.5[b]	7.5±0.8[b]	6.3±0.7[b]	8.1±0.9[b]
Selected	11.6±1.1[a]	11.5±0.9[a]	8.2±0.9[a]	12.3±1.3[a]
sig	**	*	*	**

NF, non fertilised pasture; FP, fertilised pasture. **P*≤0.05; ***P*≤0.01; ****P*≤0.001; ns, non significant difference.

The lamina length of tillers/plants was greater in grasses species (*L. perenne* and *B. valdivianus*) in sowing pastures compared with FP and NFP where the lamina length of *L. nudicaulis* was greater (Table 8). The species selected were greater than non selected. The lamina length on pastures was similar between the fertilised pastures during Autumn, Winter and Spring, and significantly greater than NFP (Table 9). In Summer, lamina length of Ln was greater than Lp and Bv (Table 9).

Table 9. Lamina length (cm) of tillers/plant from different type of pasture (Mixture, Diverse, non-fertilised pasture [NFP] and fertilised pasture [FP]), species (*L. perenne*, *B. valdivianus* and *L. nudicaulis*) and defoliation selected (nonselected and selected) in each season.

	Autumn	Winter	Spring	Summer
Mixture	10.4±1.1[a]	9.8±0.9[a]	12.4±1.3[a]	10.5±0.8[ab]
Diverse	10.8±0.8[a]	9.8±0.8[a]	11.3±1.4[a]	9.9±1.3[b]
NFP	5.2±0.4[b]	4.3±0.4[b]	6.3±0.4[b]	6.3±0.7[c]
FP	10.9±0.9[a]	8.4±0.6[a]	13.2±0.8[a]	11.9±1.2[a]
sig	*	*	**	***
Lp	10.7±0.9[a]	9.0±1.2[a]	12.7±0.6[a]	7.7±1.1[b]
Bv	10.2±0.8[a]	7.1±0.8[b]	12.3±0.7[a]	9.1±0.8[b]
Ln	6.3±0.5[b]	8.3±0.4[a]	6.5±0.4[b]	11.6±0.8[a]
sig	*	*	*	*
Non selected	9.4±0.4	8.4±0.4[b]	10.5±0.4	8.6±0.7
Selected	10.5±0.8	11.3±0.6[a]	9.1±0.8	11.1±0.6
sig	ns	**	ns	ns

Lp, *L. perenne*; Bv, *B. valdivianus*; Ln, *L. nudicaulis* *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns, non significant difference. Values on the same column followed by the same letters present significant differences.

3.5 Nutritive value of the evaluated species across seasons

The nutritive value (CP, NDF and WSC) of the species throughout the season are presented in Figure 4. There were interactions between species and season (*P*≤0.001) for CP, NDF and WSC. Pasture with fertiliser applied (Mixture, Diverse and FP) had a higher CP content than NFP. The significant interaction (*P*≤0.05) between species an season showed that there were no differences between *L. perenne* and *L. nudicaulis* during Spring and Summer. In Autumn and Winter, CP was greater in *L. perenne*.

For NDF, it was lowest in NFP compared with fertilised pasture. In both pastures, NFP and FP, NDF was similar between *L. perenne* and *B. valdivianus* (*P*≥0.05) and greater than *L. nudicaulis* (*P*≤0.05) during Autumn, Winter, Spring and Summer.

The water soluble carbohydrate content was higher in FP than NFP. In NFP, *L. perenne* had a higher WSC content than *B. valdivianus* and *L. nudicaulis* during Spring and Summer. In Winter, *L. perenne* and *B. valdivianus* were similar (*P*≥0.05) and greater than *L. nudicaulis* (*P*≤0.05). In FP, *L. perenne* had a higher WSC content in all seasons compared with *B. valdivianus* and *L. nudicaculis* (*P*≤0.001). The lowest WSC content was registered in *B. valdivianus* during Autumn and Spring (*P*≤0.05). In Winter and Sumer the lowest content was in *L. nudicaulis* (*P*≤0.05) compared with *L. perenne* and *B. valdivianus*.

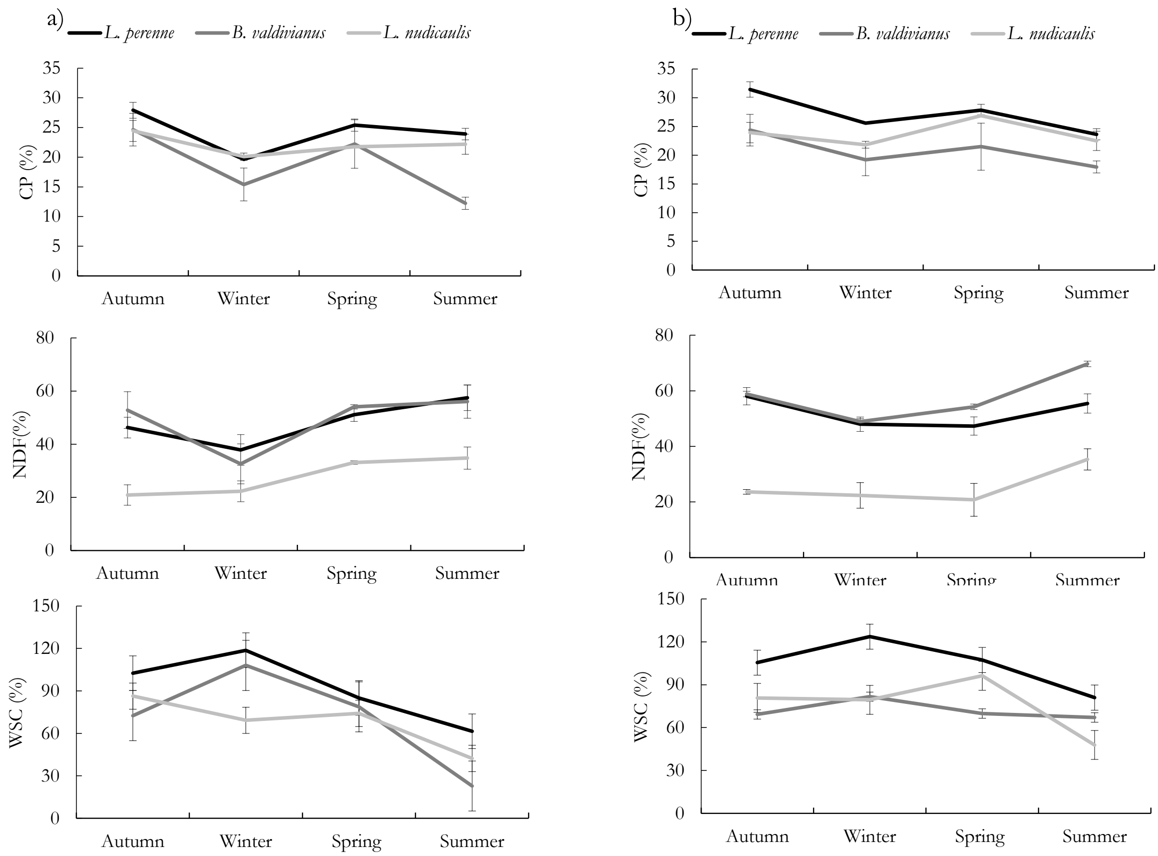

Figure 4. Interaction between species and season on crude protein (CP), neutral detergent fibre (NDF) and water-soluble carbohydrate concentration (WSC) of a) NFP and b) pastures with fertilised applied (Mixed, Diverse and FP). Error bars indicate standard error of differences for species x season interaction.

The principal component analysis showed the relationships between the nutritive value of the species and morphological traits with the species score vectors (*L. perenne*, *B. valdivianus* and *L. nudicaulis*) (Figure 5a) and pastures types score vectors (Figure 5b). In the Figure 5a, the first two dimensions resume 81.8% of the total variance of dataset (PC1 63.7 and PC2 18.1). The first component (PC1) related the morphological traits (pre grazing lamina length and lamina weight offered) with *B. valdivianus* and *L.perenne*. The second component (PC2) distinguished the nutrient content (CP and NDF) and in the opposite post grazing lamina length, WSC with *L. nudicaulis*.

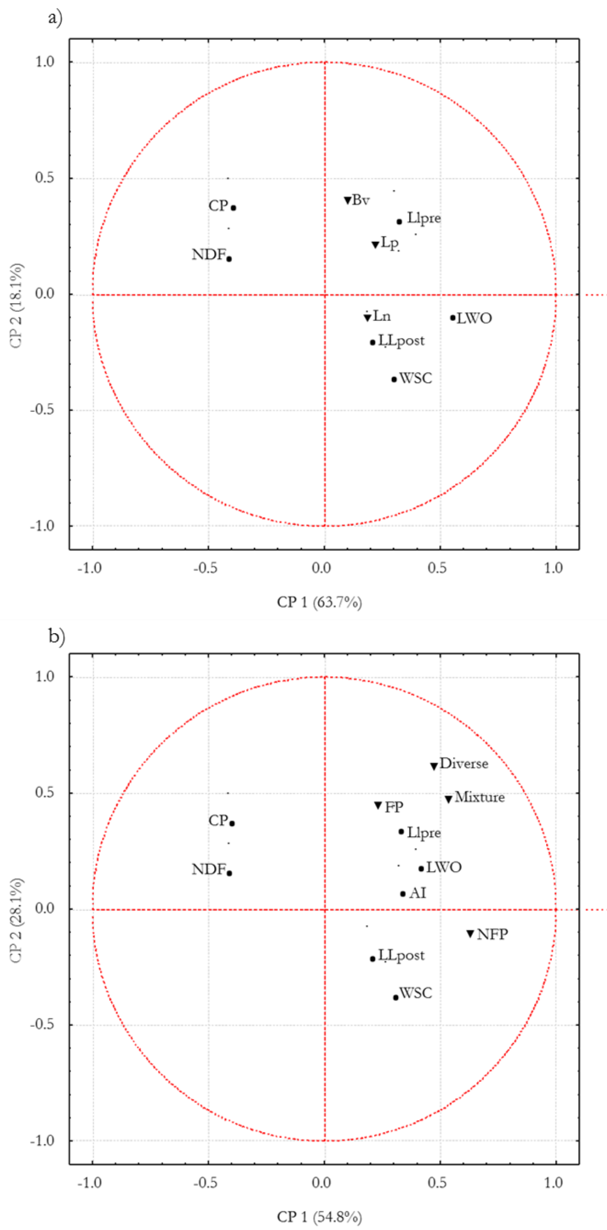

Figure 5. Plot of the first two PC loading vector describing the relationships between nutrient value variables of species and morphological variables with a) the species (Lp, *L. perenne*; Bv, *B. valdivianus* and Ln, *L. nudicaulis*, and b) with the pastures types (Mixture, Diverse, NFP and FP).

Crude protein (CP), neutral detergent fibre (NDF) and water-soluble carbohydrate concentration (WSC), pre grazing lamina length (Llpre), post grazing lamina length (LLpost) and lamina weight offered (LWO), Apparent intake (AI). (▼) Score vectors; (●) Loading vectors.

The figure 5b, the 82.9% of the total variance was explained by the first two components (PC1 54.8% and PC2 28.1%). The first component (PC1) shows the separation of morphological traits (pre grazing lamina length, lamina weight offered and apparent intake) with Diverse, Mixture and FP. The second component (PC2) distinguished the nutrient content (CP and NDF) and in the opposite, NFP was related with post grazing lamina length and WSC.

4. DISCUSSION

During the evaluation period, pastures had to survive to two consecutive dry Summers (Figure 1). The Summer of the first year had three months of water restriction (January to March). The Summer of the second and third year, the drought period was from December to April.

The improvement of a naturalised pasture revealed the importance of fertiliser and lime addition towards decreasing the soil fertility limiting factors to pasture growth, and also shows the relevance of grazing control to the pasture improvement process. Both, fertilisation and grazing control are responsible of providing the favourable conditions to increase pasture herbage mass (Malhi et al 2011; Keim et al 2014). As a result of the soil fertility improvement, pasture botanical composition changed towards increasing the high fertility tolerant species (*L. perenne*, *B. valdivianus* and *H. lanatus*).

4.1 Pasture productivity, botanical composition and month daily growth rate

On the first year, FP produced 8% (535 DM ha^{-1} $year^{-1}$) more than Diverse. The higher DM yield on FP during the first year may be explained by the fact that Diverse was sown at the end of Summer. Therefore, this pasture had to establish before accumulating herbage mass; meanwhile, FP was already growing during the establishment time of the sown pasture.

Considering the three years of evaluation, FP showed possitive effects of managements and fertiliser, achieved 62% (6612 kg DM ha^{-1} $year^{-1}$) more than NFP. Due to FP has more available soil nutrients as consequence of the fertilization and liming, the species can use the environmental resources (temperature, water and soil) and getting their potential growth.

The botanical composition reflects the increase of soil nutrient availability, one of the main factors interfering on pasture production. There were an increase of grasses species such as *L. perenne*, *H. lanatus* and *B. valdivianus*, while NFP maintained its original species composition conformed by species tolerant to soil acidity such as *A. capillaris* and flatweeds.

In this study, the increase of legume content under fertiliser application was more than 100% in Mixture, Diverse and FP. For *Trifolium repens* Mixture registered from 434 to 1343 kg DM ha^{-1} $year^{-1}$, Diverse from 1289 to 1719 kg DM ha^{-1} $year^{-1}$ and FP fron 60 to 1130 kg DM ha^{-1} $year^{-1}$. On the other hand, NFP reduced the proportion of *T. repens* from 704 to 143 kg DM ha^{-1} $year^{-1}$. The increase of legume species founded in this study were contradictory with other experiments. Lambert et al. (1986) found that a low application of N (<50 kg N ha^{-1} $year^{-1}$) was enough to reduce *T. repens* in 10%. However, there are other soil nutrients, such as the P and K that stimulates legumes growth in the pasture. This was the case of Aydin and Uzun (2005) which after applying 52 kg P ha^{-1} $year^{-1}$ to a pasture conformed of 38% legumes, 35% grasses and 27% flatweeds, the contribution of the legumes increased from 3500 kg DM ha^{-1} $year^{-1}$ to 5000 kg DM ha^{-1} $year^{-1}$ (42.8%).

4.2 Nutritive value of the pastures

The increased of CP and SP in Mixed, Diverse and FP could be attributed to high proportion of green leaves (Bryant et al., 2012) and also has been associated to the leaf nitrogen content when plants are fully grown (Ayden and Uzun, 2005). In NFP, the increase of CP, especially during the Spring time, it is likely the resulted from the enhancement of soil activities such as organic matter mineralization (Cookson et al., 2005; Tian et al., 2010). The increase of metabolizable energy and WSC content could be used as a resource to initiate new growth of perennial grasses (Turner et al., 2007). Pasture species such *L. perenne* and *B. valdivianus* increase the WSC content on the pastures (Turner et al., 2007; 2010) and species such *D. glomerata* decrease the content of WSC (Turner et al., 2006).

4.3 Selective grazing

During the second year of pastures improvement, selective grazing was evaluated in the fertilised pastures (Mixture, Diverse and FP) and NFP. In this period, *L. nudicaulis* was the dominant species in FP and NFP, while *L. perenne* and *B. valdivianus* were dominant in the sown pastures (Mixture and Diverse). *Lolium perenne* and *B. valdivianus* have similar foliage mass and nutritive value (Muñoz, 1980; López et al., 2013). As flatweed, *L. nudicaulis* has rosette structure to ground level. Soder et al. (2009) registered a possitive relationship between plant species and pasture height in the species selection as was found in this study. The sheep use their lips and teeth as a feeding tool (Arnold, 1966). The lower lips move away from the teeth allowing to bite closer to the ground and selecting species in the pasture (Dwyer, 2009).

During Autumn and Spring, the probability of a specific plant being grazed by the sheep was significatively greater for *L. perenne* and *B. valdivianus* than for *L. nudicaulis*. The quantity of the grazed marked plants being consumed was also significantly greater for *L. nudicaulis* than *L. perenne* and *B. valdivianus* in both seasons. While in Summer, *L. nudicaulis* was selected more than both grasses. In NFP, the sheep selected *L. nudicaulis* over the grass species in all seasons. These results show that sheep do actively selects species to be grazed when the species increases its availability in the pasture.

In Summer, pasture growth rate decreases due to water stress, thus sheep expand their diet in favor to species such as *L. nudicaulis*. One consequence of this is that pasture probability *L. nudicaulis* being consumed increases. In addition, flowering stem density increased in *L. nudicaulis* being often preferentially consumed by sheep (Augustine and Frelich, 1998; Mulder and Ruess, 1998). In Winter, *L. perenne* and *L. nudicaulis* were selected over *B. valdivianus* because both species had similar leaf length.

There is a close relationship between lamina length and lamina weight of *L. perenne* to that reported by Hodgkinson et al. (2010), with correlation coefficients (R) of 0.86. The relationships for *B. valdivianus* and *L. nudicaulis* for lamina length and lamina weight has not been previously published. In these study a strong relationship was found between these two variables for *B. valdivianus* and *L. nudicaulis* with correlation coefficients (R) of 0.76 and 0.81, respectively. This information was used to calculate the amount consumed of the marked tiller/plants. The amount of the marked tiller/plant that were apparently consumed were greater in Spring for *L. perenne* and *B. valdivianus*, and greater in Summer for *L. nudicaulis*. The lamina length of *L. perenne* and *B. valdivianus* was similar in all pastures and it was greater than *L. nudicaulis* lamina length in Autumn, Winter and Spring. *Leontodon nudicaulis* presented a significantly longer lamina length during Summer because *L. perenne* and *B. valdivianus* reduced their lamina length in response to water stress (López et al., 2013).

4.4 Nutritive value of *L. perenne*, *B. valdivianus* and *L. nudicaulis*

During the Spring, *L. perenne* registered a high CP content, which had a close relationship with the increase of grazing probability. NDF always was superior in grasses species and WSC evidence a wide variation in *B. valdivianus* and *L. nudicaulis*. However, there was positive relationship between the grazing probability of *L. nudicaulis* with WSC and lamina length postgrazing. For *L. perenne* and *B. valdivianus*, the grazing probability was associated with lamina length pregrazing.

The higher probability of the *L. nudicaulis* plants of being grazed in Summer was due to the increase of *L. nudicaulis* which may have enough WSC to be palatable. Also, the lamina weight offered explained that high consumption in *L. nudicaulis*. For *L. perenne* and *B. valdivianus*, the selection in Autumn and Spring is associated with regular input of fertilisers, providing the nutrients to achieve and maintain the tillering in both species (Chapter 2). The regular input of fertiliser improves the species environment to growth, and as consequence the species developed more tillers and greater lamina length. The selective grazing can be indirectly associated with the fertilization because the sheep selectively grazed through the pastures according to the herbal mass production. Also, animal grazing stimulates tiller regrowth (Evju et al., 2006).

The data reported in this study can be directly transferred to field applications in relation to pasture management, improvement and grazing control. Lime, fertiliser application and sheep grazing on naturalised degraded pasture had positive response on pasture productivity and succession of botanical composition. The grass species were preferably selected by sheep with a subsequence tillering increase, however, under stress conditions as water stress, the selection pressure was predominantly over the flatweeds.

5. CONCLUSION

Fertilization and liming in a naturalised degraded pasture improved the botanical composition through the increase of high fertility tolerant species, resulting in a pasture production and nutritive value similar to sown pastures.

Sheep actively select the species of plants to be grazed, with a marked preference for *L. perenne* and *B. valdivianus* in fertilised pastures and when herbage mass increased. When herbage mass was low and under water stress conditions sheep increased the grazing selection in *L. nudicaulis*. There is a positive relationship between lamina length and grazing, with the animals selecting larger laminas.

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Chapter 2

Tillering dynamics and competition between *Lolium perenne* L. and *Agrostis capillaris* L. in a natural degraded pasture as response to fertilisation

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ABSTRACT

The sustainability of pasture ecosystem depends on the increase of soil nutrients when pasture condition is improving. The aim of this study was to evaluate the effect of fertilizer addition on tiller population dynamics and competition between *Loium perenne* and *Agrostis capillaris* on a naturalised degraded pasture. Species competition was evaluated on a naturalised no fertilised pasture (NFP) and on a naturalised fertilised pasture (FP). Metal rings were fixed to the soil in each pasture containing: 100% *A. capillaris* tillers (Ac), 100 % *L. perenne* tillers (Lp) or a mixture of both species in a proportion of 50% *A. capillaris* – 50% *L. perenne* (Acm-Lpm). Tiller population was counted in each ring every 15 days. The botanical composition and nutrient content of the pastures were measurement through the experimental period. FP had higher levels of crude protein, water soluble carbohydrates, neutral detergent fiber and metabolisable energy than NFP. *Lolium perenne* increased in the pasture with fertiliser addition as part of the botanical composition, which explained the increment of the pasture accumulated herbage mass. Fertilised pastures had more tillers than the unfertilised with a significant interaction between fertilisation and species ($P \leq 0.05$). Lp and Ac had significant differences in autumn and summer in the first year, while in the second year the differences between Lp and Ac was in autumn ($P \leq 0.05$). In mixture, Lpm and Acm had significant differences in autumn and spring only in the first year. Thus, increasing the availability of soil nutrients due to fertiliser addition, increased tiller density and population of *Lolium perenne* over *Agrostis capillaris*. Therefore, Lp behaves as better competitor than Ac and becomes dominant in the pasture when soil fertility does not constraint its growth.

Keywords: naturalised pasture, sustainable pasture improvement, soil nutrient, tiller population

1. INTRODUCTION

A large proportion (48%) of the naturalised pastures in the south of Chile are not properly managed, i.e. are overgrazed, thus their pasture production is low (Balocchi, 1999) and exist different levels of soil degradation affecting negatively pasture growth, botanical composition and nutritive value (Tharmaraj et al., 2008). Strategies for proper pasture management have being applied to improve soil nutrient availability, pasture production and persistency. Fertilisers are applied to correct or reduce soil nutrient deficiencies as nitrogen, phosphorus and potassium, being nitrogen the main limiting factor for temperate pasture growth once the soil fertility levels have been corrected (Fang et al., 2007).

Pasture species have different abilities to extract and utilize nutrients (Perkins et al., 2011), which result in differences in plant growth and tillering (Cecato et al., 2001; Roma et al., 2011). The increase of soil nutrient availability affects positively the pasture community structure and composition (Stevens et al., 2004) towards fast growing species colonization (Stevens et al., 2004; Liancourt et al., 2009), and the exclusion of slow growing species and less invasive perennial species (Poorter et al., 1995; Shaver et al., 2001).

In humid temperate regions and under fertile conditions species such as *Lolium perenne* L., are widely used to renovate degraded pastures (Gastal et al., 2010). It is characterised by its fast establishment, strong ability for tiller develop (Tharmaraj et al., 2008) and high dry matter production (Deak et al., 2007). In contrast, not fertilised pastures are usually dominated by slow growing species as *Agrostis capillaris* L., which is a stress tolerant perennial grass, adapted to harsh environmental conditions and low soil fertility (Zhao et al., 2006). Grazing stimulate tillering of *L. perenne* causing an opposite effect on others species such as *Festuca arundinacea* Schreb. in pastures grased by sheep and cattle (Stewart and Charlton, 2006).

When a pasture achieves significant proportions of fast growing species the application of fertiliser may be useful to improve pasture production through the increase on tiller population (Butkuviene and Butkute, 2008; Matthew et al., 2013). In general, the responses after fertiliser and lime application are well understood (Poozech et al., 2010) and pasture usually changes its botanical composition (Pennings et al., 2005). However, insufficient fertiliser inputs and mismanagement of weeds may contribute to the poor persistence of fast growing species (Nie et al., 2004). These features suggest that strategic management of pasture, fertiliser and lime addition should affect positively faster growing species. Faster growing species may be more competitive and may have better ability to retain dominance after fertilisation displacing stress tolerant species.

This study investigated the hypothesis that the fertiliser addition on a naturalised degraded pasture, allows spontaneous establishment and development of *Lolium perenne* L., which by competition displaces to *Agrostis capillaris* L. Pasture improvement processes were evaluated on a naturalised degraded pasture with and without fertiliser, measuring tillering dynamics and competition of the slow growing stress tolerator species (*A. capillaris*) and fast growing competitor species (*L. perenne*). Also, herbage mass production and botanical composition were evaluated.

2. MATERIAL AND METHODS

2.1 Study area

The study was carried out at Austral Agricultural Research Station of Universidad Austral de Chile (39º 47' 26" S, 73º 14' 12" W) 9 km east of Valdivia city, Chile. The experimental site was located at 25 m a.s.l., and 2350 mm of average annual rainfall. The soil corresponded to a Duric Hapludand (IREN et al., 1978).

2.2 Treatments

Two pastures were evaluated: naturalised non fertilised pasture (NFP) and naturalised fertilised pasture (FP), from May 2011 to May 2012. Pastures layout was organised in a randomised complete block design with three blocks (plots with an area of 400 m^2) for each pasture. FP received an annual fertilisation of 180 kg N ha^{-1} $year^{-1}$, 120 P_2O_5 ha^{-1} $year^{-1}$, 120 kg K_2O ha^{-1} $year^{-1}$ and 2 t ha^{-1} $year^{-1}$of $CaCO_3$ as lime.

Each pasture was grazed by twenty five 2-years old sheep, Austral breed, live weight 55 ± 2.42 kg (average ± SEM) and body condition 2.71± 0.27 (average ± SEM), when it reached an equivalent of 2.100 to 2.300 kg DM ha^{-1} until 1.000 to 1.200 kg DM ha^{-1} (Matthews et al., 2004). The maximum length of defoliation interval was 60 days. Botanical composition was measured (May 2011 and May 2012) in both pastures (Table 1).

2.3 Soil and pasture measurement

Daily temperature and soil water content were measured by sensors placed 10 cm deep in the soil (Figure 1). Soil fertility level was determined from April 2010 in each sampling plot. In each sampling plot, 60 soil samples were collected randomly (0-20 cm depth) in order to make twelve composed samples (each sample a composite of 5 sub samples). Whole soil samples were air dried to determine pH, organic matter, soil mineral nitrogen, Olsen P, aluminium saturation following Sadzawka et al. (2006) method. Soil mineral nitrogen was measured as $N–NO_3$ and $N–NH_4$ using KCl as extract and Kjeldahl digestion method (Bremner, 1996). Olsen P was determined by extraction with sodium bicarbonate (Olsen, 1954).

Table 1. Percentages of botanical composition (DM bases) in samples collected in May 2011-2012.

	2011		2012	
Plant species	NFP	FP	NFP	FP
Grasses				
Lolium perenne L.	3	3	0	8
Bromus valdivianus L.	13	5	5	4
Holcus lanatus L.	11	6	18	22
Agrostis capillaris L.	5	34	34	24
Anthoxanthum odoratum L.	2	4	6	7
Dactylis glomerata L.	4	*	1	*
Festuca arundinacea Schreb.	*	*	*	3
Legumes				
Trifolium repens L.	13	1	10	5
Trifolium subterraneum L.	*	*	*	*
Lotus uliginosus Schkuhr.	*	*	*	1
Flatweeds				
Leontodon nudicaulis L.	15	25	22	17
Cirsium vulgare (Savi) Ten.	*	1	*	1
Rumex acetosella L.	*	*	*	*
Taraxacum officinale Weber.	9	15	2	6

*value < 1%

Quality herbage was estimated in both treatments cutting all pasture above 4 cm to soil level in areas randomly chosen. The samples were dried at 60 °C for 48 hrs in a forced air oven until constant weight to determine Crude protein (CP; Micro Kjeldahl method; AOAC, 1995), soluble protein (SP; Licitra et al., 1996), neutral detergent fibre (NDF; van Soest, 1967) and acid detergent fibre (ADF; AOAC, 1995) and digestible organic matter in the dry matter (DOMD, Tilley and Terry, 1963). Metabolizable energy (ME) was calculated from DOMD using the Garrido and Mann (1981) equation: ME (Mcal/kg DM) = $0.279 + 0.0325 \times$ DOMD.

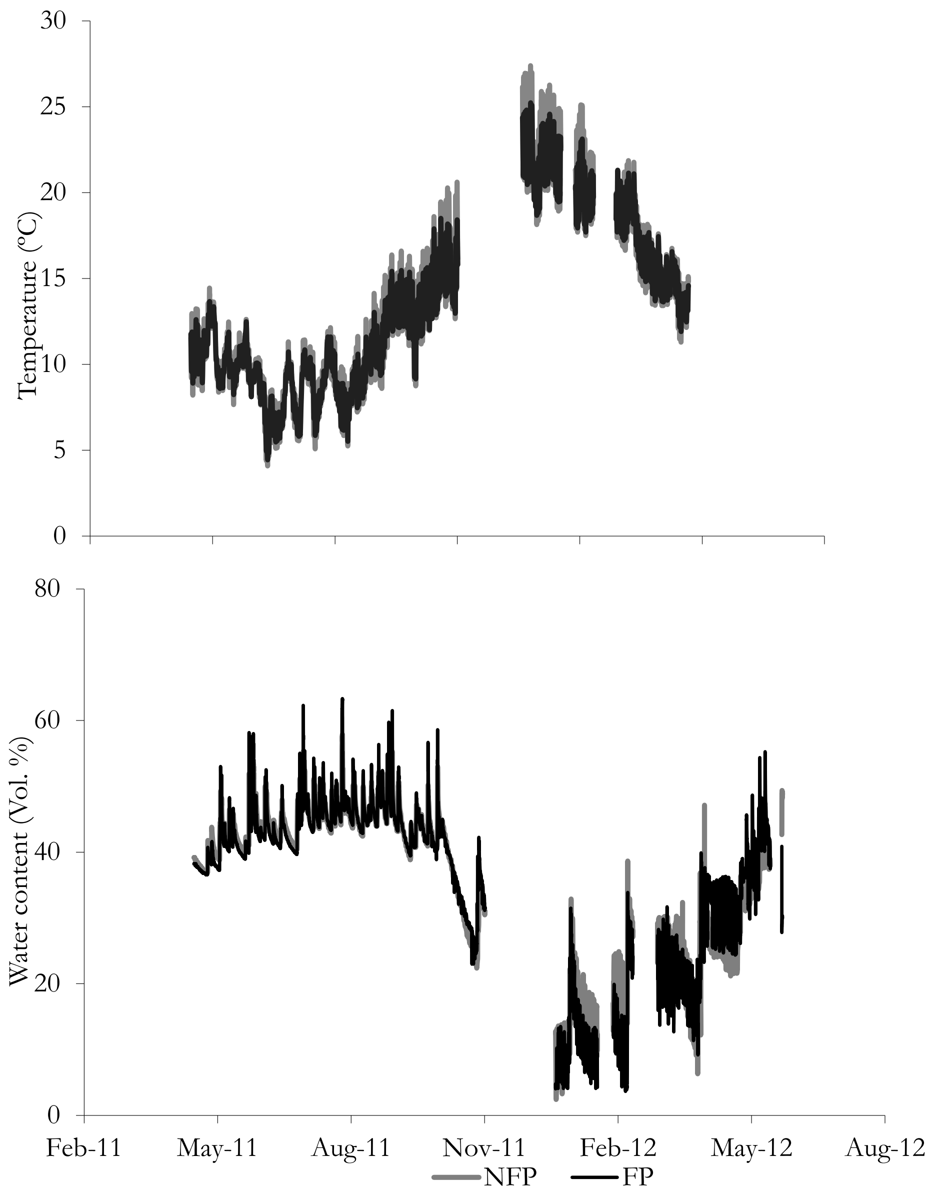

Figure 1. Temperature and water content during the study

2.4 Tiller dynamics treatments

The tiller dynamics was measured using modificated method from Bahmani et al., (2003). Nine metal rings (6 cm diameter) were placed in each experimental plot, on the 26 may 2011. Rings containing 100% *A. capillaris* tillers (Ac), 100% *L. perenne* tillers (Lp), and a mixture of both species (50% *A. capillaris* tillers (Acm), 50% *L. perenne* tillers (Lpm)) with 3 rings for each class. The numbers of live tillers within each ring was counted every 15 days.

Dry matter of *L. perenne* and *A. capillaris* grown under the treatment combinations (100% and 50%) were measured through the tiller length.

2.5 Statistical analysis

All statistical analyses were conducted using the GLM procedure of the program SAS, Version 12.3 (SAS Institute Inc, 2011). Treatments effects were analysed according to a 3x2 randomized block design with factorial arrangement of the treatment, corresponding to 3 rings of each class of pasture (100% *A. capillaris* tillers, 100% *L. perenne* tillers, and a mixture of both species) and 2 levels of fertilisation (NFP, FP). To determine statistical differences between treatments Analysis of variance (ANOVA) and LSD means comparisons were applied (Steel et al., 1997).

The statistical model was:

$$y_{ijkl} = \mu + \beta_i + \alpha_j + \tau_k + \alpha\tau_{jk} + \varepsilon_{ijkl}$$

Were:

y_{ijkl} = response dynamics tiller growth and competition evaluated variables between species

μ = the overall mean

β_i = block effect

α_j = three types of rings effect

τ_k = fertilisation effect

$\alpha\tau_{jk}$ = treatments interaction

ε_{ijkl} = random error

3. Results

3.1 Soil and pasture measurement

The highest soil water content was reached between May and August, when soil temperature was close to 5 °C in its first 10 cm. This period of the year is characterised by increasing winter precipitation and the soil will be almost completely saturated. After a rainfall, soil water content decreases due to rapid drainage, as a result of the high internal permeability of this type of soil (Dörner et al., 2012). Soil water content decreased from September onwards, reaching the lowest values between December and February, while soil temperature increased over 26 °C (Figure 1). However, from October to February the soil was wetted and dried repeatedly due to intermittent rainfalls throughout this period.

Soil fertility improved due to fertilizer and lime application by diminishing soil aluminium saturation ($P \leq 0.01$), increasing total soil exchangeable bases ($P \leq 0.01$), especially exchangeable potassium and sodium ($P \leq 0.05$) (Table 2). There were differences between NFP and FP for accumulated pasture production (Figure 2) with a positive response in FP to fertilisation.

The pasture nutritive value (CP, SP, ME, WSC and NDF) is presented in Table 3. There were no statistical differences ($P \geq 0.05$) among the treatments for CP content during the Spring of the first year with values from 218 to 236 g kg^{-1} DM. During the second year, FP had the highest CP content ($P \leq 0.05$) and NFP the lowest. In Autumn and Winter ($P \leq 0.05$) FP had the highest CP content. Statistical differences in the SP content of the pasture were found in all seasons (*P*≤0.05). During Autumn (*P*≤0.01) and Spring (*P*≤0.05) of the first year, FP registered the highest content in SP, while in the second year the highest values was in FP during Winter (*P*≤0.01). There were significant differences among the treatments for the ME content in all seasons with highest values in FP. In the first year the highest values were registered in Autumn and Winter (*P*≤0.01). During Autumn (*P*≤0.05) of the second year (*P*≤0.05) FP showed the highest value in ME content. The pastures showed differences in their WSC content only in Autumn (*P*≤0.01) on the first year. During the first year, FP showed the highest content in NDF during Autumn (*P*≤0.05) and Summer (*P*≤0.01) than NFP.

Table 2. Soil fertility in original soil (April 2010) and experimental soils

Soil Deph: 20 cm		April 2010	April 2011		April 2012		
		Original soil	NFP	FP	NFP	FP	sig
pH water	(1:2.5)	5.6	5.5	5.7	5.6	5.9	ns
pH CaCL2 0.01 m	(1:2.5)	4.8[b]	4.9[b]	5.0[b]	4.9[b]	5.3[a]	*
Soil Organic Matter	(%)	14.4	14.4	13.8	15.2	14.6	ns
Olsen P	(mg/kg)	9.3	9.7	10.1	9.4	11.5	ns
Soil Exchangeable Potassium	(mg/kg)	68.0[b]	70.3[b]	142[a]	63.2[b]	135[a]	*
Soil Exchangeable Sodium	(cmol/kg)	0.13[b]	0.06[b]	0.09[b]	0.13[b]	0.22[a]	*
Soil Exchangeable Calcium	(cmol/kg)	1.93[b]	1.72[b]	2.75[b]	2.92[b]	6.41[a]	***
Soil Exchangeable Magnesium	(cmol/kg)	0.47[cd]	0.45[d]	0.65[b]	0.63[bc]	0.97[a]	***
Total Exchangeable Bases	(cmol/kg)	2.7[b]	2.4[b]	3.9[b]	3.8[b]	7.9[a]	***
Soil Exchangeable Aluminium	(cmol/kg)	0.38[a]	0.38[a]	0.22[bc]	0.29[ab]	0.10[c]	**
Aluminium Saturation	(%)	12.2[ab]	13.9[a]	5.9[cd]	7.9[bc]	1.6[d]	**
Available Sulphur	(mg/kg)	13.0[a]	11.8[ab]	12.3[ab]	6.6[c]	9.9[b]	**

NFP No fertilised pasture and FP Fertilised pasture * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$.

Figure 2. Accumulated herbage mass (kg DM ha^{-1}) of non fertilised pasture (NFP) and fertilised pastures (FP) during first and second evaluation year

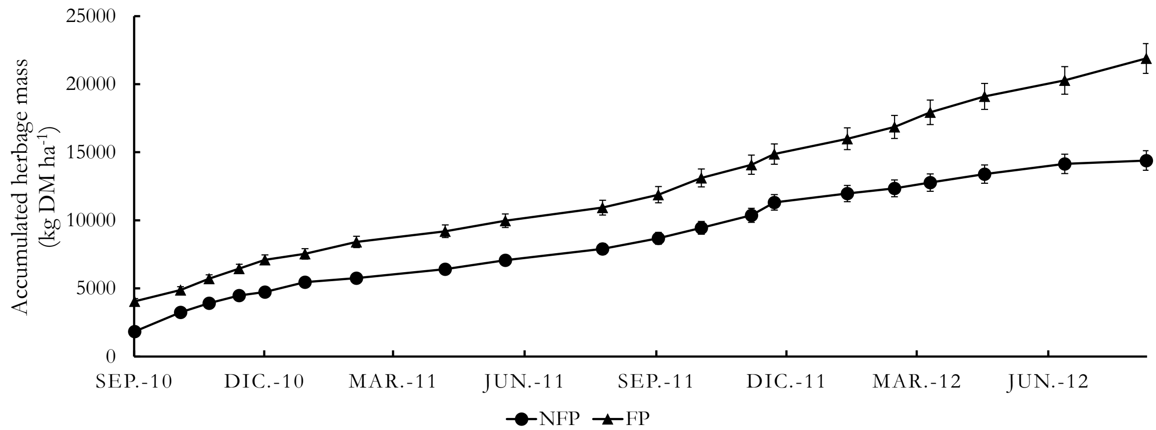

Table 3. Effect of fertilizer application on the improvement of pasture nutrient content of a naturalized degraded pasture with and without fertiliser through the season.

	2011				2012	
	Au	Wi	Sp	Su	Au	Wi
Crude Protein (g kg^{-1} DM)						
NFP	206[b]	224[b]	218	133	210[b]	219
FP	261[a]	251[a]	236	172	229[a]	229
sig	**	**	ns	ns	*	ns
Soluble Protein (g kg^{-1} DM)						
NFP	66b	70[b]	66[b]	52	96[a]	88
FP	88[a]	86[a]	88[a]	78	85[b]	92
sig	**	*	*	ns	*	ns
Metabolizable Energy (MJ kg^{-1} DM)						
NFP	10.3[b]	10.5	9.5[b]	8.3	9.2[b]	10.5
FP	10.8[a]	10.8	10.5[a]	8.7	10.9[a]	9.8
sig	**	ns	*	ns	*	ns
Water Soluble Carbohidrates (g kg^{-1} DM)						
NFP	66[b]	77	53	73	80	88
FP	87[a]	93	60	76	85	93
sig	**	ns	ns	ns	ns	ns
Neutral Detergent Fibre (g kg^{-1} DM)						
NFP	310[a]	291[a]	519	409[b]	405[b]	480
FP	325[b]	263[b]	517	545[a]	491[a]	473
sig	*	**	ns	**	**	ns

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$; NFP non fertilised pasture; FP Fertilised pasture.

3.2 Tiller population density

Over the whole evaluation the fertilised pasture showed a higher tiller population density than the unfertilised pasture with significant interaction between fertilisation and species ($P \leq 0.05$). FP showed highest tiller density compared with NFP through the seasons ($P \leq 0.05$). Lp and Ac had significant differences in Autumn and Summer in the first year, while in the second year the differences between Lp and Ac was in Autumn ($P \leq 0.05$). In mixture, Lpm and Acm had significant differences in Autumn and Spring only in the first year (Table 4).

Table 4. Seasonal tiller population density (tillers/m^2) in non fertilised pasture (NFP) and fertilised pasture (FP).

	2011				2012	
	Autumm	Winter	Spring	Summer	Autumm	Winter
NFP	12821^b	11483^b	12041^b	7260^b	9820^b	13706^b
FP	18251^a	16453^a	19031^a	10765^a	11832^a	14195^a
sig	*	*	*	*	*	*
Lp	13577^b	14157^a	15741^a	6845^b	8026^b	14060^a
Ac	17494^a	13778^a	15330^a	9786^a	10447^a	14088^a
Lpm	7699^c	7092^b	10530^b	3041^c	4404^c	6026^b
Acm	6532^d	5857^b	6373^c	3323^c	4952^c	6627^b
sig	***	***	***	**	**	*
NFPLp	12293^d	12400^c	14563^d	7903^c	8378^d	12576^b
FPLp	26617^b	29507^a	27883^a	13396^b	14921^b	23614^a
NFPAc	25145^c	20548^b	17408^c	13329^b	19963^a	23260^a
FPAc	31771^a	28028^a	25992^b	19172^a	11975^c	11840^b
NFPLpm	7787^e	7965^d	8467^e	3480^e	4545^f	5730^d
FPLpm	7612^e	13094^c	5717^{fg}	2601^f	4261^f	6323^c
NFPAcm	6057^f	7251^e	5494^g	4326^d	6392^e	8258^c
FPAcm	7006^{ef}	5494^f	6219^f	2318^f	3458^f	4996^e
sig	***	***	***	**	*	**

Lp 100% *Lolium perenne* L.; Ac 100% *Agrostis capillaris* L.; Lpm 50% *Lolium perenne* L.; Acm 50% *Agrostis capillaris* L.* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$;

3.3 Tiller dynamics growth

Figure 3 shows the growth dynamics of the tiller population of Lp and Ac, growing as monoculture or mixture, with or without fertilizer addition, from May 2011, to August 2012. Without fertiliser addition, tiller population of 100% Lp had a stronger increased during the first Spring in relation to 100% Ac, but after that period the tiller population of both species grew in a similar rate. In FP, Lp growing as a monoculture had higher tiller population development than Ac, especially from the second half of winter towards the Spring. This behavior was consistent during both evaluated years.

When both species were grown as mixture under non fertilised conditions, Lp tiller population density had a higher increased on tiller number than Ac during the Spring time. The tiller population of both species was similar along the Summer. Under fertilised conditions, Lp was a better competitor than Ac, as its tiller population increased faster during Spring, Autumn and Winter.

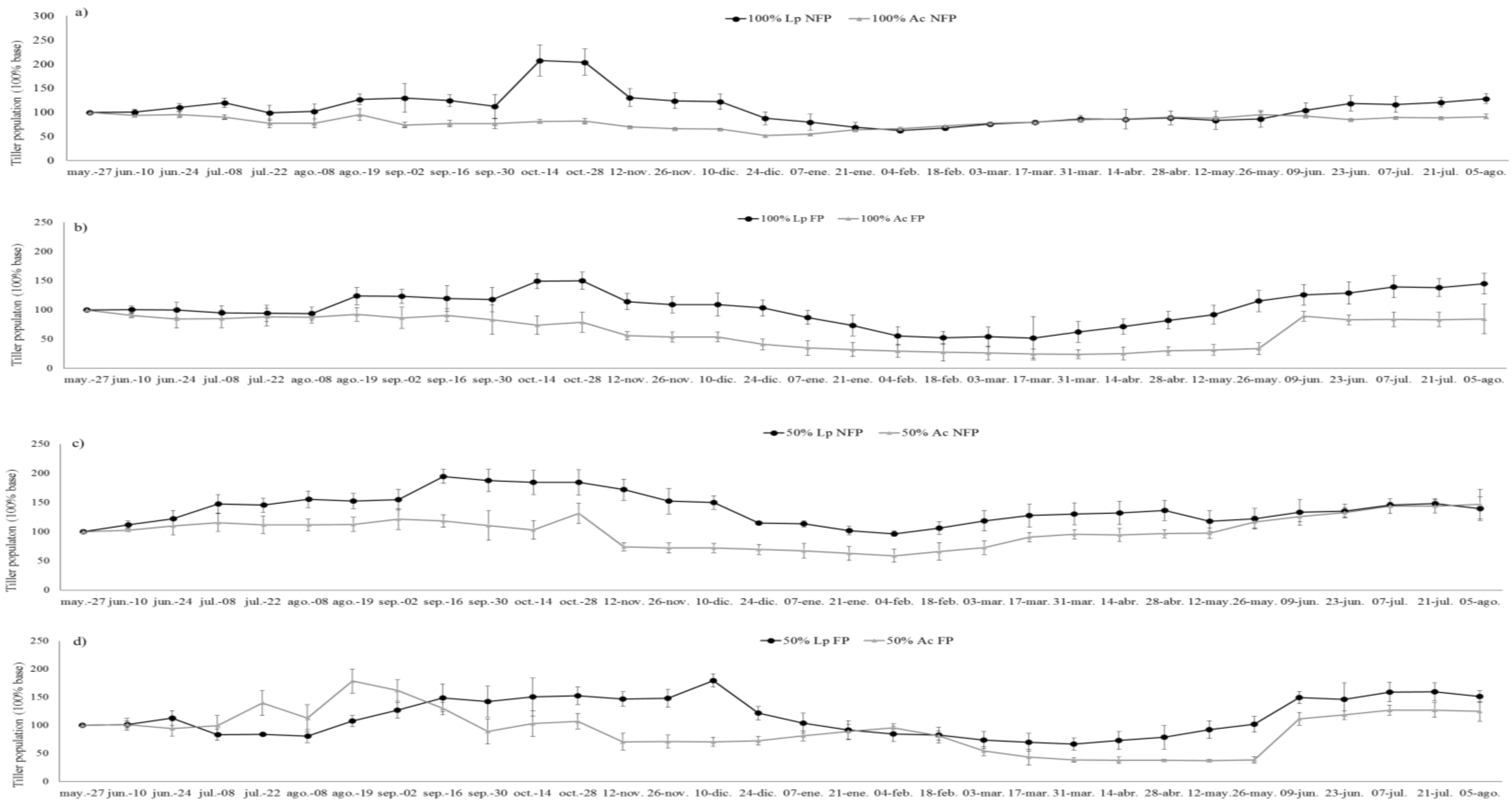

Figure 3. Tiller population dynamics (100% base) of *Lolium perenne* (Lp) and *Agrostis capillaris* (Ac) in non fertilised pasture (NFP) and fertilised pasture (FP). Lp 100% *Lolium perenne* L.; Ac 100% *Agrostis capillaris* L.; Lpm 50% *Lolium perenne* L.; Acm 50% *Agrostis capillaris* L.* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$

The dry matter of both species responded differently to treatment interactions. In NFP, Lp had a significant decrease in biomass compared with FP (Figure 4). In Fp, Lp had an increase in biomass with the increase of soil nutrients and had the largest values in competition with Ac.

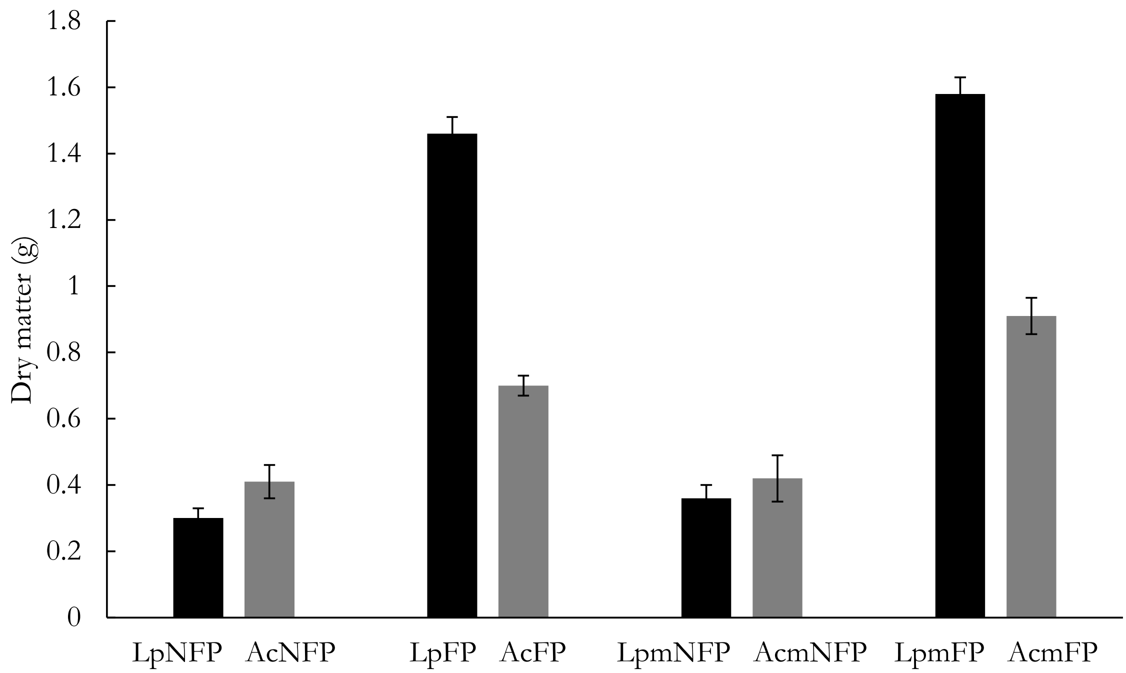

Figure 4. Mean (±1 SE) dry matter of *Lolium perenne* (Lp) and *Agrostis capillaris* (Ac) grown under all treatments combinations as: (LpNFP) 100% Lp or (AcNFP) 100% Ac without fertilizer; (LpFP) 100% Lp or 100% Ac with fertilizer; (LpmNFP) 50% Lp and (AcmNFP) 50% Ac without fertilizer; (LpmFP) 50% Lp and (AcmFP) 50% Ac with fertiliser.

4. DISCUSSION

4.1 Botanical composition and soil nutrients

The tiller population density was according with the usual pattern of growth fast growing species on pastures with fertiliser and lime regime (Bahmani et al., 2003; Hirata and Pakiding, 2004; Sbrissia et al., 2010). This is the consequence of competition for soil nutrients, due to the increase of soil nutrients availability, which is one of the main factors interfering in the development of new tillers (Hermans et al., 2006). Gatti et al., (2013) registered under N fertilisation a positive response by *Bromus willdenowii* which produced 81% more tillers than *Dactylis glomerata* and 21% more leaves in competition with N fertilisation.

At the beginning of the evaluation occurred changes on species plant composition. Changes in the botanical composition were registered in the second year of fertiliser applied (2012). These changes showed the increase of grasses species such as *L. perenne*, *H. lanatus*, *A. odoratum* and *F. arundinacea*, which have a favorable effect in accumulated pastures production (Chelariu et al., 2007; Chelariu, 2008). Positive lime and fertiliser response have been observed by Brum et al., (2009) with the increase on 64% of grasses species on pastures. The difference in accumulated pasture production might be related to dominant species. NFP reflected the original soil condition formed by slow growing species such as *A. capillaris*, *T. officinale* and *L. nudicaulis*. These species are characterised by support stress tolerant constraints (Bernier and Alfaro, 1996). In FP, fertilisation and liming reduced or maintained the proportion of fast growing species, which might indicate that the increase of accumulated pasture production in FP occurred as a result of positive growth of species such as *L. perenne*.

NFP and FP, showed different responses to soil nutrients availability in terms of tiller population density. High values of tiller population were recorded in FP during the first year (2011). As long the experiment progressed, tiller population values for both pastures decreased during Summer and Autumn, starting to increase again during Winter on the second year (2012). This reflected the seasonal pattern of pasture production throughout the year explained because between end of Winter and early Spring, grasses started to grow when soil temperatures increase (4°to 6°C by Robson et al., 1988) and the application of N at this time of the year promotes a faster stem elongation, pasture growth and maturation of grass leaves on species such as *L. perenne*, *D. glomerata* and *F. arundinacea*, whereas other species decreased herbage production (Brum et al., 2009) as here with *A. capillaris*.

Water soil deficit and high soil temperature have being associated to tiller diminishment (Sbrissia et al., 2010). The water soil deficit and high soil temperature could have have generated a mechanism to reduce tiller population in order to ensure plant persistence until environmental conditions are favorable. For example, the increase of leaf area index by the plant allows to maintain a number of tiller enough to stay alive (Sbrissia et al., 2010). Also, the plants have de ability to lead (increase or decrease) the tiller population density as mechanism that optimizes their resistance to defoliation regime and light interception (Matthew et al., 2013). In Summer, persistence and survival of *L. perenne* is difficult due to the seasonal water restriction that generates a low soil water availability (López et al., 2013). Consequence of that is the high rate of dead tillers that happen during that period (Sbrissia et al., 2010). The lower growth of tiller population density in Summer, did not affect tiller turnover in the next seasons. Both species, *L. perenne* and *A. capillaris*, had high tiller turnover in Spring and a diminishment during Summer (Gastal et al., 2010).

Lolium perenne had the tendency to be dominant the most part of the year with a recover in tillering in Autumn and an acceptable Winter growth. Mathew (1992) showed in *L. perenne* cv. Ellet some activity still able to produce new tillers. The response of the species toward soil nutrients improvement was highly predictable in line with the colonization - competition model of Grime (1974). In the present study, the higher dry matter (g) of *L. perenne* under N supply (100% Lp and Lpm) was reflected by N uptake capacity as consequence to lamina length. Gastal et al. (2010) found high tiller population density of *L. perenne* and *D. glomerata* under high N supply determined by its higher specific N ability. Also they showed, high relative growth rate of *Festuca rubra* under low N supply with a significantly higher N concentration in root and shoot tissues. In this study, *A. capillaris* (100% Ac and Acm) had a dry matter production similar to *F. rubra* under low soil nutrients overpassing Lp. With these results, both species demonstrated their capacity to be plastic under changes in soil nutrients availability.

4.2 Competition and tiller dynamics

In competition and independently if fertiliser was applied, *L. perenne* always showed an overcome to *A. capillaris*, suggesting that competition by nutrients was the main factor affecting *L. perenne* tillering, whereas the *A. capillaris* maintain its higher stress tolerance ability, keeping a constant number of tillers through the year. The nitrogen fertilization causes protein synthesis and thus an increase in vegetative growth (Xu et al., 2012) resulting in the increase of *L. perenne* tillering. The tillering was supported by carbohydrates reserves which are used to protein synthesis (Hermans et al., 2006; Xu et al., 2012). *L. perenne* could present a content of water soluble carbohydrates (WSC) highest than *A. capillaris* achieving a high tiller population promoted by fertiliser and, more strongly when water and other nutrients such as potassium are available (Megda and Monteiro, 2010). Nitrogen fertilisation stimulate the synthesis of amino acids and amide compounds to detriments of carbohydrates reserves because carbohydrates reserves are used for protein synthesis (Miller et al., 2008).

There was a positive linear relationship between plant water soluble carbohydrate concentration, leaf DM and tillers regrowth per plant after defoliation in grasses species (Rawnsley et al., 2002). In *Dactilis glomerata*, during regrowth, an accumulation of WSC reserves coincided with a resumption of tillering and root regrowth (Rawnsley et al., 2002). Donaghy and Fulkerson (1998) showed that tillering is related to water soluble carbohydrates reserves in the plant. In the open canopy situation, sunlight stimulates the tillering trough WSC as current photosynthate, whereas with defoliation it is the high stubble WSC reserves that initiate tillering (Donaghy and Fulkerson, 1997; Lee et al., 2007).

The low N supply produces stunted plants which have small cells and reduces lamina elongation rate (Kavanová et al., 2008). In grazed pastures the low N supply can result in a patchwork pattern of growth development due to high N return by the animals that enhance pasture patchiness. The remainder of the pasture shows poorer growth with less grass dominance and more legumes species (Tonzer et al., 2011). In unfertilised pastures, grass species present more carbohydrates reserves than protein maintaining a lower plant growth. *A. capillaris* was adapted to low N availability soil and continuously will be forming new tillers at a low growth rate, while, *L. perenne*, had a pattern of tiller turnover (Sbrissia et al., 2010) with a high tillering rate specially when nitrogen was applied as management practice on Spring and Autumn.

The regular input of fertilizers provide nutrients to achieve and maintain *L. perenne* tillering. The strategic applications of fertiliser encourage pasture growth at specific time and also, in combined nutrients forms such as N, P, K and S could ensure the establishment of grass species like *L.perenne*. The interspecific competition between *L. perenne* and *A. capillaris* leading to practical management and fertiliser addition, is a key factor to improve the vigour of desirable species (Tonzer et al., 2011) such as *L. perenne* and reduce species which dominate in low fertility conditions.

5. CONCLUSION

The increases of soil nutrients availability through fertiliser increase the tiller density and population of *Lolium perenne* L. over *Agrostis capillaris* L. In these conditions, Lp is better competitor and dominant than Ac. Thus, fertilization and liming of a degraded pasture is an alternative for pasture improvement in humid temperate region.

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Chapter 3

Relationship between lamina length and tillering population of *Lolium perenne* L. and *Agrostis capillaris* L. in natural degraded pasture under different light regimes

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ABSTRACT

Temperate grass species under different light regimes can have diverse plant growth, tiller density, lamina morphology and crude protein (CP). The aim of these study was evaluate tillering dynamics and lamina characteristics of *Lolium perenne* L. and *Agrostis capillaris* L. in a natural degraded pasture under different intensities of light regimes. Three regimes of light incidence were calculated in relationship with photosynthetic active radiation (PAR) as follow: 60.7, 140.5 and 199.9 μmol $m^{-2}s^{-1}$ and measured through shade structures covers with mesh which represented Heavy shade (HSh), Medium shade (MSh) and Open sunlight (OS), respectively. Each shade structure were placed in 3 blocks of natural degraded pastures (NFP) and natural fertilised pastures (FP). Tiller dynamics were measured in six metal rings (6 cm diameter) placed in the pastures under each structure. The rings (2 rings for each class) contained 100% *A. capillaris* tillers (Ac), 100 % *L. perenne* tillers (Lp) or mixture of both species: 50% *A. capillaris*-*L. perenne* (Acm-Lpm). The CP, CCI, leaf area and lamina length increased as shaded increased with the highest values registered in FP for HSh (by *L. perenne*) and the lowest in NFP for OS (by *A. capillaris*). In FP, Lp and Lpm had a stronger increased in MSh and HSh, while, Ac was higher than Lp on Summer. In NFP, Ac tiller dynamics had a highest increased in OS and HSh compared with MSh where Lp was dominant.

Keywords: leaf area, light restriction, tiller, chlorophyll

1. INTRODUCTION

The environment of a pasture, as defined as climate and soil (White and Hodgson, 2000) has an important influence on pasture growth. The soil mineral nutrients, water, temperature and radiation have been studied towards to improve pasture growth (Dood et al., 2005) analyzing for agronomical possibilities for reaching pasture potential growth (Thajmaraj et al., 2008; Dekvota et al., 2009; Sbrissia et al., 2010; López et al., 2013).

The soil mineral nutrients and the increase of soil fertility were used to improve natural degraded pastures (Fang et al., 2007) and to increase the proportion of fast growing species, such as *Lolium perenne* L., widely used to renovate degraded pastures (Gastal et al., 2010). It is well known that pasture improvement is strongly affected by management factors, as well as grazing regime and the use of nitrogen fertiliser (Kemp et al., 2014). Tiller population density of *L. perenne* in natural fertilised pastures was larger than slow growing species, such as *A. capillaris*, after three years of N fertiliser applied under sheep intensive grazing (Chapter 2). Similar results were obtained over *Poa pratensis* by Tallec et al., (2008) indicating that it was a poor competitor for nutrients.

In fertile environments, light competition by plants is probably more important than nutrient or water competition (Tilman, 1994). The number of tillers per plant and the rate of tiller appearance have been related to the intensity of photosynthetically active radiation (PAR), for example, *Triticum aestivum* (Bos, 1999) and *Lolium perenne* (Bahmani et al., 2000), especially in nearly closed canopies (Simon and Lemaire, 1987). The sunlight radiation that plant leaves are receiving is an important variable for plant growth (Zhu et al., 2010). The light quantity (incident solar radiation) and light quality (light spectrum) affect pasture productivity and plant development (Chory, 2010; Kami et al., 2010; Li et al., 2012), thus the plant captures the photosynthetic active radiation of the sunlight energy and use it to generate its carbohydrates through the photosynthesis process (White and Hodgson, 2000).

Light environment can modify leaf growth and tiller appearance. It has been shown that self-shading within a dense canopy at the tiller base is associated with increased lamina length and decreased tillering (Kays and Harper, 1974; Devkota et al., 1998; Wherley et al., 2005; Lemaire et al., 2009; Byrt, 2011). These effects were linked by Casal et al. (1985, 1987) with low red:far ratio associated with shading which induced an increase in leaf area through increased lamina elongation rate and leaf elongation duration (Allard et al., 1991), compensating for the lower light interception (da Silveira Pontes et al., 2010) . Also, shaded plants usually have thinner and wider lamina than unshaded plants (Wahl et al., 2001; Zhai et al., 2006). The effect of high sward leaf area index in inhibiting tillering (Simon and Lemaire, 1987) is an important mechanism controlling tiller density, and lamina length is the major component of pasture leaf area index.

Differences in the response of grass species to shade in terms of plant growth, morphology and nutritive value have been reported earlier (Wahl et al., 2001; Bahmani et al., 2000; Peri et al., 2006; Devkota et al., 2009). However, there is limited information on the effects of a range of incident light over pastures in grazing systems.

This study investigated the hypothesis that the fertiliser addition and the reduction of incident radiation on a naturalised degraded pasture, allows the development of *Lolium perenne* L., which by competition displaces to *Agrostis capillaris* L., increasing its tillering and leaf area. The aim of the study was to evaluate the competition between *Lolium perenne* L. and *Agrostis capillaris* L. as affected by light intensity modifying tillering dynamics and lamina growth when both species are a part of an improving natural degraded pasture.

2. MATERIAL AND METHODS

2.1 Study area

The study was located at the Santa Rosa Research Station of Universidad Austral de Chile (39º 47’ 26” S, 73º 14’ 12” W), Valdivia, Chile. The experimental site was located at 25 m a.s.l., and 2350 mm of average annual rainfall. The soils are classified as Duric Hapludand Soils (IREN et al., 1978). The monthly rainfall and average temperature during the experimental period (October 2012-August 2013) are shown in Figure 1.

Figure 1. Monthly precipitation and mean temperature values during the experimental period

2.2 Treatments

Two pastures were evaluated, a naturalised non fertilised pasture (NFP) and a naturalised fertilised pasture (FP). Pastures layout was organized as a randomised complete block design with three replicates (plots with an area of 400 m^2) for each pasture. Each pasture treatment was grazed with twenty five 2-year old sheep, Austral breed, live weight 55 ± 2.42 kg (average ± SEM) and body condition 2.71± 0.27 (average ± SEM), once it reached an average pre-grazing herbage mass of 2.100 to 2.300 kg DM ha^{-1} until 1.000 to 1.200 kg DM ha^{-1} (Matthews et al., 2004). The maximum length of defoliation interval was 60 days.

Fertilisers were applied annually to achieve a pasture production of 9443 kg DM^{-1} $ha^{-1}year^{-1}$ in FP (180 kg N ha^{-1} $year^{-1}$, 120 kg P_2O_5 ha^{-1} $year^{-1}$, 120 kg K_2O ha^{-1} $year^{-1}$ and 2 t ha^{-1} $year^{-1}$ of $CaCO_3$ as lime). NFP registered 5854 kg DM^{-1} ha^{-1} $year^{-1}$.

In October 2012 botanical composition in NFP was composed by 34% *Leontodon nudicaulis*, 1% *Lolium perenne*, 4% *Bromus valdivianus*, 5% *Holcus lanatus*, 3% *Trifolium repens*, 39% *Agrostis capillaris* and 41% of flatweeds. While FP was composed by 34% *Leontodon nudicaulis*, 9% *Lolium perenne*, 10% *Bromus valdivianus*, 7% *Holcus lanatus*, 6% *Trifolium repens*, 10% *Agrostis capillaris* and 38% of flatweeds.

2.3 Experiment design and shade structures

Within each plot, shade structures covers with mesh were used to reduce light incidence to three levels of photosynthetic active radiation (PAR µmol $m^{-2}s^{-1}$): 60.7 Heavy shade (HSh), 140.5 Medium shade (MSh) and 199.9 Open sun light (OS). The measurements of these structures were: 30 cm height, 70 cm length, 100 cm width for HSh and 20 cm height, 65 cm length, 75 cm width for MSh. The position of the shade structures was South-North.

2.4 Environmental measurements

The volumetric water content (VWC) in the top of 10 cm (Dec et al., 2012) was measured every 15 days with time domain reflectometry (TDR) (Time Domain Reflectometry; TDR 200 Soil Multimeter FOM/mts, easy Test, Lublin, Poland.). The photosynthetic active radiation (PAR) as photosynthetic photon flux intercepted in μmol $m^{-2}s^{-1}$ was measured at ground level and above canopy height in each shade structure, every 15 days with SKP 116 (400-700 nm; 600-800 nm) 'Standard' Fibre Optic Light Measuring System; Skye, Powys, UK. The measured PAR where taken between 11:00 h and 13:00 h.

2.5 Pasture species measurements

The crude protein (CP) was measured on a Leco CNS Analyzer (Leco CNS-2000, St Joseph, Michigan, USA) which is based on the Dumas dry combustion technique. The analyses was determined every season. Lamina length was measured every 15 days and prior animal grazing (if that coincided) using a metal ruler in the lamina of 10 vegetative tillers (from the tips of its ligules) growing in the rings placed under each structure. In the same lamina, chlorophyll content index (CCI) was determined used a digital chlorophyll meter CCM-200 Plus (OptiSciences, Tyingsboro, Mass.). The measurements were recorded in the adaxial leaf side facing the sunlight (Cerovic et al., 2012). For each species, leaf area (n= 100) were measure using a leaf area meter (LI-3100C, LI-COR, Lincoln, NE, USA) once time per season.

2.6 Tiller dynamics

The tiller dynamics was measured using the modificated method from Bahmani et al., (2003). Six metal rings (6 cm diameter) were placed in the pasture under each structure and fixed to the soil (October 2012). The rings (2 rings for each class) contained 100% *A. capillaris* tillers (Ac), 100 % *L. perenne* tillers (Lp) or mixture of both species: 50% *A. capillaris-L. perenne* (Acm-Lpm). The numbers of live tillers within each ring was counted every 15 days.

2.7 Statistical analysis

All statistical analyses were conducted using SAS (version 12.3, SAS Institute Inc., 2011). Treatments effects were analysed according to a 3x3x2 randomized block design with factorial arrangement of the treatment, corresponding to 2 rings of each class of pasture (100% *A. capillaris* tillers, 100% *L. perenne* tillers, and a mixture of both species), 3 levels of shade (HSh, MSh and OS) and 2 levels of fertilisation (NFP, FP). To determine statistical differences between treatments, Analysis of variance (ANOVA) and LSD means comparisons were applied (Steel et al., 1997). Soil moisture, crude protein, chlorophyll, lamina length and leaf area were analysed separately for each species with analysis of variance (ANOVA) and PDIFF means comparisons were applied (Steel et al., 1997).

3. RESULTS

3.1 Soil water content

The daily average temperature and precipitation were measured at open sunlight. The highest temperature and the lowest precipitation (mean of 20.7 °C and 4.32 mm, respectively) were registered in early Summer (December). The lowest temperature for the experimental period was recorded in June (average temperature 5.6°C) and the highest rainfall was in July (337.3 mm month^{-1}). During the whole evaluation period, MSh and HSh registered higher soil water content (VWC) than OS for the top 10 cm of soil. In Autumn and Winter FP HSh and FP MSh registered above 34%, while NFP HSh and NFP MSh, 32% of VWC. The lowest soil

VWC was registered in early Summer (December) with an average 13.6% in FP and 14.8% in NFP (Figure 2).

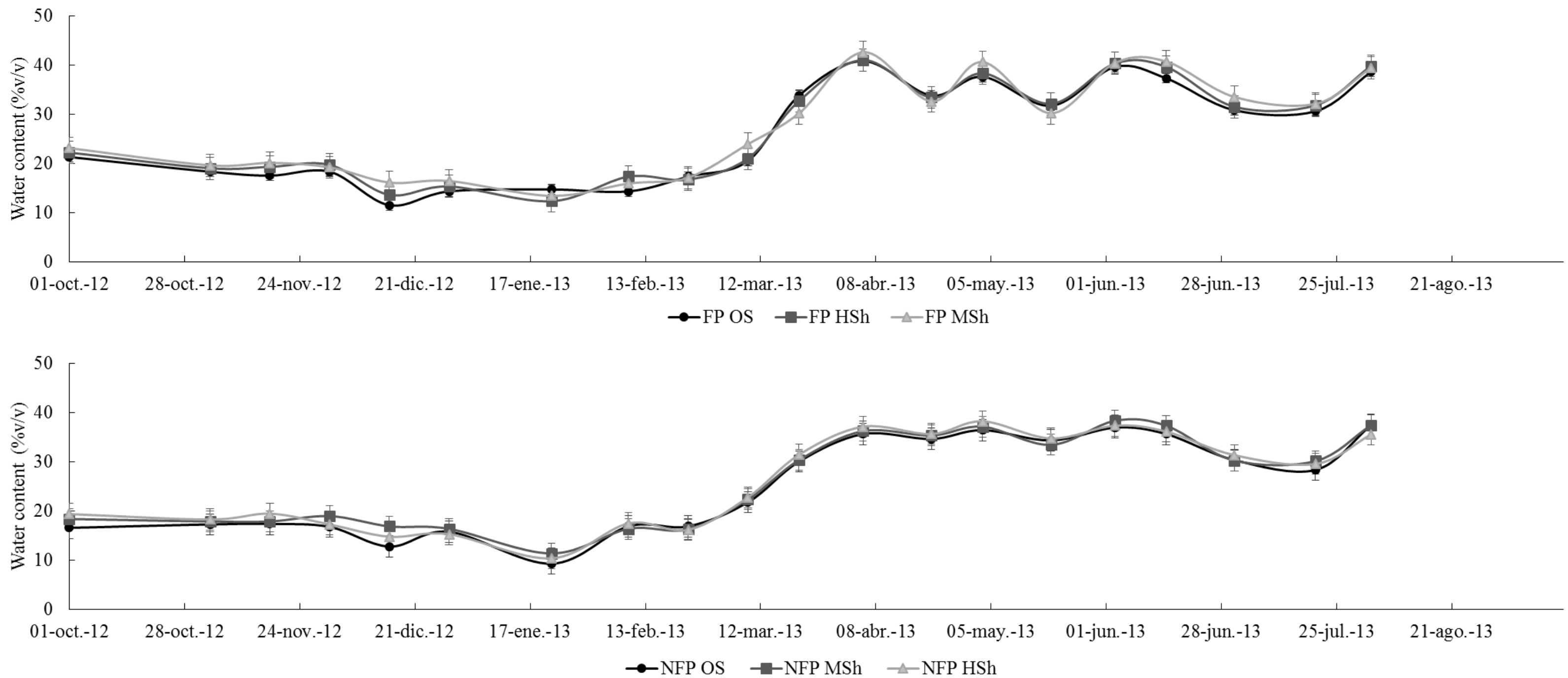

Figure 2. Soil volumetric water content 10 at cm depth under three levels of shade. FP, Fertilised pasture; NFP, No fertilised pasture; OS, Open sunlight; HSh, Heavy shade; MSh, Medium shade.

3.2 Daily photosynthetic active radiation (PAR)

The daily photosynthetic active radiation (PAR) was used to calculate the accumulated monthly photosynthetic photons per unit area (Figure 3). The maximum photosynthetic photons daily levels reached in FP and NFP were in January for OS (189.9 μmol $m^{-2}s^{-1}$), while the minimum photosynthetic photons daily levels were measured in HSh (4.5 μmol $m^{-2}s^{-1}$). The pastures in the open sunlight received 20.1 and 47.3 μmol $m^{-2}s^{-1}$ more than MSh and HSh.

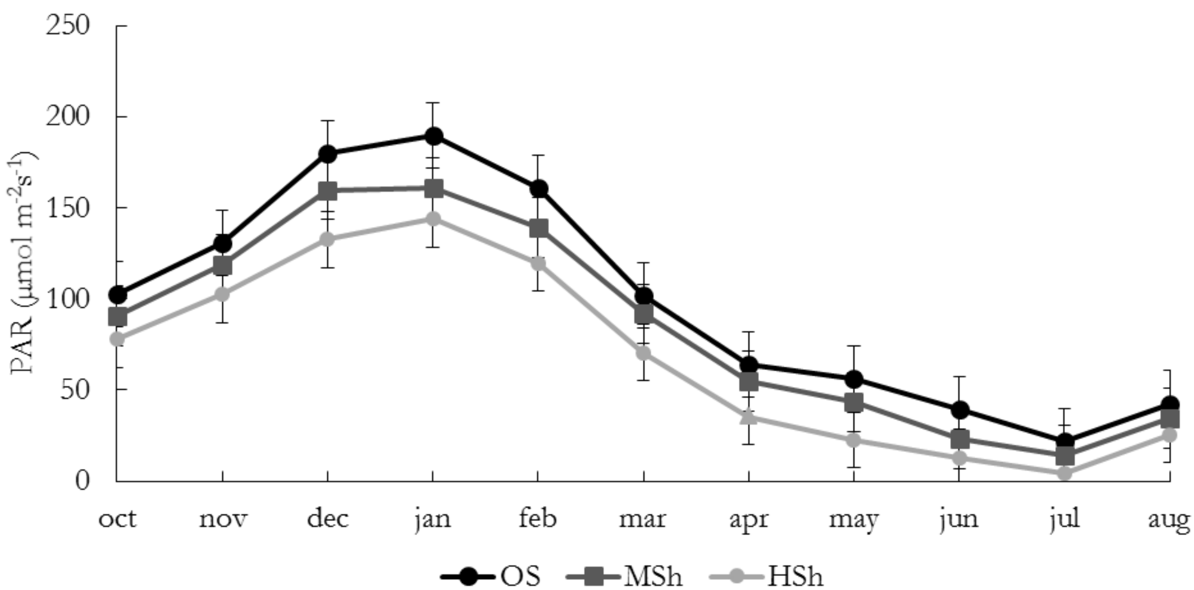

Figure 3. Mean monthly photosynthetic active radiation (PAR) (400–700 nm waveband) on the three levels of shade. HSh, Heavy shade; MSh, Medium shade; OS, Open sunlight.

The daily PAR in the open sunlight for a sunny day in Spring (solar angle elevation 53.4°), Summer (solar angle elevation 67.4°), Autumn (solar angle elevation 45.7°) and Winter (solar angle elevation 32.2°) were used as reference to calculate transmissivity of shade structures (Table 1). The solar angle elevation was used to represent the relative reduction of photosynthetic photons in shaded treatments compared with open sunlight. The total photosynthetic photons received in OS on Summer (29th December) was 183 x 10μmol $m^{-2}s^{-1}$, which was 4 times higher than in Winter (20th July) (Table 1). The transmissivity on shaded structures decreased with the diminishment of the solar angle elevation from Summer to Winter.

Table 1. Transmissivity of the shaded treatments as a percentage of the photosynthetic active radiation (PAR) for sunny days at three different solar angle elevation. The total daily PAR for open shaded structure (μmol $m^{-2}s^{-1}$) are indicated between brackets.

Solar angle at noon Treatment	53.4° Spring	67.4° Summer	45.7° Autumn	32.2° Winter
OS	100% (132.2)	100% (183.0)	100% (64.9)	100% (40)
MSh	44%	69%	40%	51%
HSh	68%	83%	78%	88%

HSh, Heavy shade; MSh, Medium shade; OS, Open sunlight.

3.3 Crude protein, chlorophyll content index and lamina length for each species

There were significant differences between pastures and structures for CP (P≤0.01), CCI (P≤0.05), leaf area (P≤0.05) and lamina length (P≤0.05). The CP, CCI, leaf area and lamina length increased as shade increased (Table 2). The highest values for all these variables were registered in FP for HSh (by *L. perenne*) and the lowest in NFP for OS (by *A. capillaris*).

Table 2. Crude protein, chlorophyll content index, lamina length and leaf area in *L. perenne* (Lp) and *A. capillaris* (Ac) in different shade structures.

	CP (%)	Chlorophyll (CCI)	Lamina length (cm)	Leaf area (cm^2)
Lp FP OS	28.01	15.17	7.87	0.41
Lp FP MSh	33.73	16.39	8.46	0.46
Lp FP HSh	37.89	17.35	9.63	0.66
Ac FP OS	26.63	13.47	6.45	0.35
Ac FP MSh	30.67	15.89	6.81	0.37
Ac FP HSh	36.23	16.37	7.19	0.36
sig	**	*	*	*
Lp NFP OS	26.09	12.39	8.25	0.45
Lp NFP MSh	26.65	12.64	7.59	0.43
Lp NFP HSh	29.78	14.49	7.34	0.40
Ac NFP OS	20.10	11.36	5.76	0.16
Ac NFP MSh	22.75	13.65	6.43	0.29
Ac NFP HSh	25.47	13.83	6.23	0.27
sig	***	*	*	*

NFP, Non fertilised pasture; FP, fertilised pasture; HSh, Heavy shade; MSh, Medium shade; OS, Open sunlight. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns $P \geq 0.05$

3.4 Tiller dynamics growth

Lolium perenne had a higher tiller number growth than Ac during the whole year (Figure 4). Shade level and fertiliser addition significantly ($P \leq 0.001$) increased the number of tiller m^{-2} within and across all dates. In FP, Lp and Lpm had a stronger increased in MSh and HSh compared with Ac. In December, January and February (Summer) Ac was higher than Lp. In NFP, Ac tiller dynamics had a highest increased in OS and HSh.

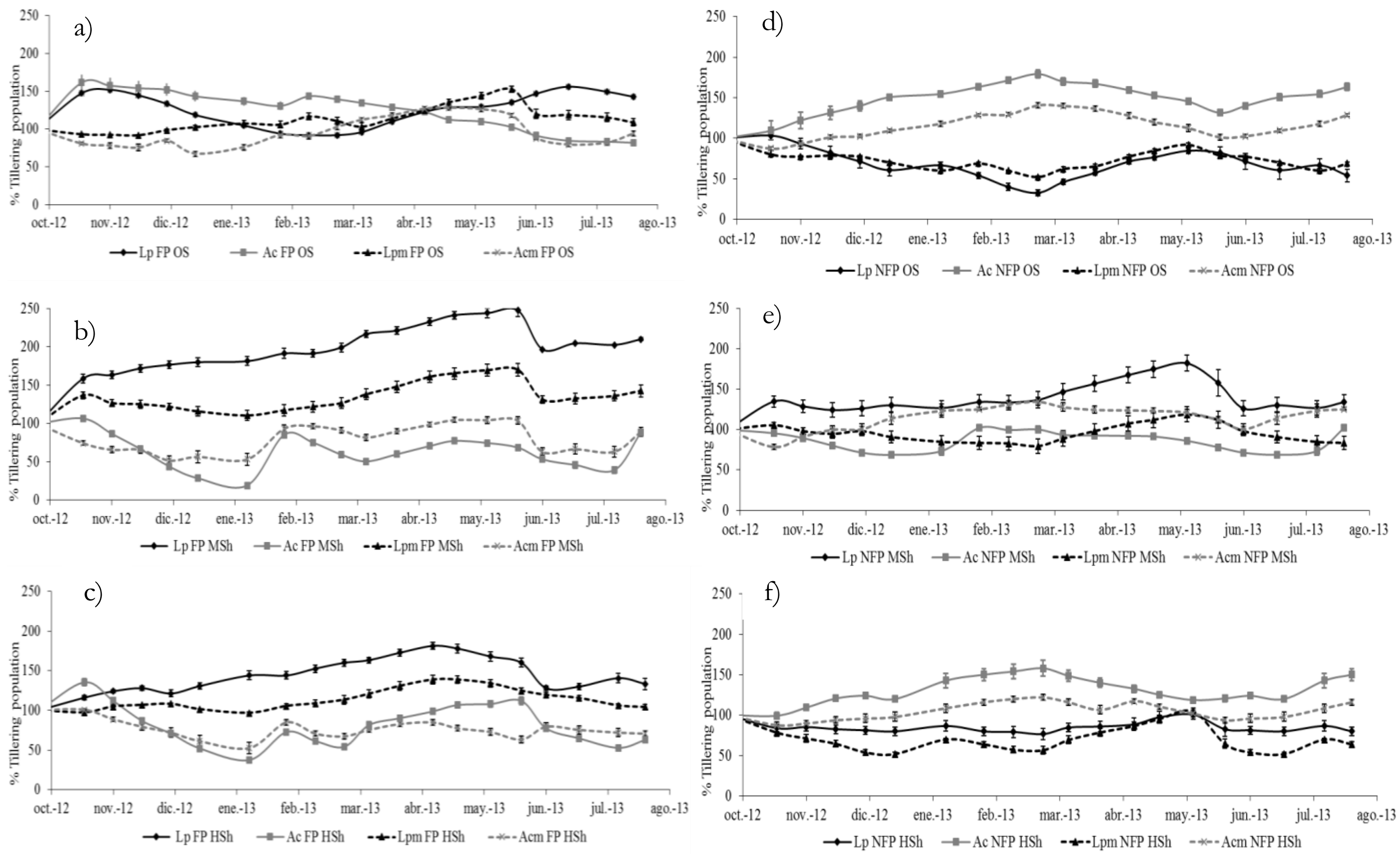

Figure 4. Tiller dynamic growth of *Lolium perenne* (Lp) and *Agrostis capillaris* (Ac) grown in fertilised pasture (FP) as: a) 100% Lp or 100% Ac, Open sunlight (OS); b) 100% Lp or 100% Ac, Medium shade (MSh); c) 50% Lp and 50% Ac, Heavy shade (HSh). Grown in non fertilised pasture (NFP) as: d) 100% Lp or 100% Ac, Open sunlight (OS); e) 100% Lp or 100% Ac, Medium shade (MSh); f) 50% Lp and 50% Ac, Heavy shade (HSh).

4. DISCUSSION

The aim of the study was to evaluate the influence of light regimes on tillering dynamics and lamina growth of *Lolium perenne* L. and *Agrostis capillaris* L. when both species were competing as part of an improving natural degraded pasture. The light regime on the pastures characterised varied between full sunlight and severe shade and it was simulated by using structures that low the sunlight that the pasture received based on the average daily PAR.

The daily PAR for a day in Summer was 183 μmol $m^{-2}s^{-1}$ (100% transmissivity) and it was reduced in MSh (70% transmissivity) and HSh (30% transmissivity). As consequence of that, tiller population rate of both pasture species decreased by 15% in MSh and 16% in HSh, compared with a 7% in OS. The reduction of DM production of *D. glomerata* due to increased shading was about 13% under slat shade, 22% under tree shade and 48% under the trees + slats shade when pasture received in open sunlight 112.5 μmol $m^{-2}s^{-1}$ (100% transmissivity) (Peri et al., 2007). Similarly, Devkota et al. (2009) incorporated trees planted to control soil erosion reporting that the tillering of *L. perenne* was suppressed by tree canopy shade (shade level between 18.3 to126.4 μmol $m^{-2}s^{-1}$) more than *Holcus lanatus*, *Dactylis glomerata* and *T. repens* thus *L. perenne* was less suitable for use in agroforestry systems. In this study, *L. perenne* was supressed when soil nutrients were limited and under HSh (shade level between 35 to 151.8 μmol $m^{-2}s^{-1}$).

The lowest Winter daily average temperature during the period of the present evaluation was ranged in July, while in December was ranged the highest Summer daily average temperature. For these periods, soil moisture content in the top of 10 cm respectively varied from 42.6% to 11.5% in FP and from 38% to 9.3% in NFP. These environmental conditions sustained tiller populations that fluctuated from 8043 tiller m^{-2} in Winter to 7955 tiller m^{-2} in Summer into FP, and from 7590 tiller m^{-2} in Winter to 7790 tiller m^{-2} in Summer into NFP. The species are capable to respond to light variations and can adapt their morphological development increasing or decreasing the tiller appearance rate (Lee at al., 2007).

4.1 Crude protein, chlorophyll content index and lamina length

Plants are frequently subjects to variations of the incident light. This is particularly of plants in the shade by developing of canopies. The proportion of incident light reaching certain plant or plant parts will be a consequence of the number of leaf layers, their disposition, and by relative contribution of radiation received (Amthor, 2010). In this study, the results indicated that a reduction in incident light was associated with longer lamina, large leaf area, high CP and chlorophyll. The longer lamina length by the decrease in light interception can be sensed by phytochrome and results in a reduction of tillering dynamic growth (Deregibus et al., 1985) as in HSh.

Lemaire & Chapman (1996) indicated that the main structural component of pasture was the leaf area index (LAI), which is sensitive to light variations and maintain a photosynthetic efficiency. The leaf area index comprises tiller population density, number of live leaves per tiller and leaf size (area) (Matthew et al., 2000). The number of leaves per tiller is relatively constant for grass species (Yang et al., 2008). Leaf size or area is a function of lamina length (Sbrissia et al., 2010) and tiller population density allows flexibility for adapting to grazing defoliation (Matthew et al., 2000). In this study, when the soil nutrients were not limited, plants responded to shading having longer leaves, may be as an effort to have a greater access to the available light while competing with neighboring plants.

The shaded conditions stimulated *L. perenne* and *A. capillaris* leaf area development to maximize light interception, resulting in longer laminas than when both species grew in OS. This is in agreement with Devkota et al., (2000) who reported that shaded plants of *D. glomerata* increased leaf area from 15.9 $mm^2\ mg^{-1}$ to 21.3 $mm^2\ mg^{-1}$. This morphologic leaf change may be corresponded to an adaptive response of phenotypic plasticity to limiting incident light (MSh and HSh), where both species showed high plasticity for lamina length associated to the increase of resource acquisition (Abraham et al., 2014).

4.2 Tiller dynamic

The reduction of light intensity did not reduce neither tiller dynamics of growth of *L. perenne* under shade, especially under MSh in FP. On the other hand, *A. capillaris* increased the tiller dynamic in OS more than in MSh and HSh especially in NFP, where expressed its ability to grow in poor soil conditions, as has been reported by Grime et al. (1987). In Winter, tiller dynamic growth of both, *L. perenne* and *A. capillaris* decreases due to low temperatures and a reduction of carbohydrates reserves. In Summer from January to April, the OS produced in FP an average of 7652 tiller m^{-2} compared with 15426 MSh tiller m^{-2} and 12027 HSh tiller m^{-2}. In NFP, the tillering number was reduced more strongly by the lack of fertiliser registering 3959 tiller m^{-2}, 10772 tiller m^{-2} and 6261 tiller m^{-2} in OS, MSh and HSh, respectively. There is some evidence that shade can benefit plants in drought periods reducing overheating, vapour-pressure deficit and oxidative stress (Sack et al., 2003). In Summer it was registered the lowest soil water content. Under shade, *L. perenne* can tolerate shade and summer drought. This tolerance was associated with a high leaf area, and high allocation to shoot than root (Sack et al., 2003). In OS, drought tolerance by *L. perenne* could be reduced by a high rate of evapotranspiration and reduced ability to capture water throughout deep roots, affecting negatively the tiller dynamic growth. The tolerance of drought plus shade may occurs as a response to soil nutrient applied, because enhance tiller production, combined with functional morphology (leaf area, reducing water loss and shade acclimation to make photosynthesis).

4.3 Tiller competition

L. perenne competing with *A. capillaris* in shaded conditions increased its tiller population. On the contrary, in OS *A. capillaris* had environmental conditions that allowed it to increase tillering over *L. perenne*. Under shade, lamina length of *L. perenne* was dominant over the lamina of *A. capillaris*. This difference between lamina lengths could be management by grazing defoliation because *L. perenne* suppressed *A. capillaris* to the upper level of the canopy through its longer leaves and increased in proportion as a result of its higher tillering rate and may have a lower tiller mortality (Garnier et al., 1999). Under continuous grazing, there is little competition for light due to the constant removal of leaf area. In contrast, under rotational grazing, competition for light increases between defoliations and the species have time to recovery carbohydrates reserves (Fulkerson & Donaghy, 2001), rebuilt shoot systems and deepen root system (Donaghy & Fulkerson, 1998).

Tillers arise from tiller buds which develop at the base of every lamina. Thus the rate of tillering is determined by both the phyllochron (Moore & Moser, 1995) and also the rate of development of the bud into a tiller. The lower tiller dynamic growth of *L. perenne* in NFP could be a result of the reduced soil nutrients in OS, but under shade, the reduction of tillering was a response to the decrease of incident light.

5. CONCLUSION

Fertiliser addition and the reduction of incident light on a naturalised pasture, allowed the development of *L. perenne* over *A. capillaris*. Under shade, *L. perenne* increased its tiller dynamic growth and leaf area. Also, *L. perenne* showed tolerance to drought under shade, increasing tiller dynamic growth. In non fertilised pasture, the decrease of tiller dynamic growth of *L. perenne* was supported by the reduction of incident light.

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Chapter 4

Prediction by decision tree modelling of the relative magnitude of functional group abundance in a pasture ecosystem in south of Chile

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ABSTRACT

Decision tree models were developed to investigate and predict the relative abundance of four functional groups of plant species [high fertility response grasses (HFRG), low fertility response grasses (LFRG), legumes and flatweeds] in a naturalized pasture in south of Chile and were compared with regression models with respect to the fitted model. In the field experiment there were 12 plots, nine of them received annual fertilizer and were grazed by twenty-five sheep from when the pastures mass reached an equivalent of 2.100 to 2.300 kg DM ha^{-1} down to 1.000 to 1.200 kg DM ha^{-1}. The maximum length of defoliation interval was 60 days. The decision tree models revealed the main factors influencing the abundance of functional groups in a humid tempered pasture in the south of Chile were Olsen P, soil mineral N and soil pH. Rainfall had secondary roles. For HFRG, soil mineral N was the most significant variable, for LFRG and flatweeds it was Olsen P, while for legumes soil pH was the most significant variable. The decision tree model performed better than the regression model with respect to model fit. Decision tree could be used in planning the fertilization of the pasture and as a tool to predict the abundance of functional groups. The successful integration of the most important variables influencing abundance of species with decision tree models provided a new approach to understanding the abundance of functional groups in humid temperated pastures and a starting point to developing managements to improve the abundance of the most productive functional group.

Keywords: decision tree; modelling; regression model; evaluation model; species abundance.

1. INTRODUCTION

Stability and sustainability of pasture in relation to the impact of management and environmental factors is a fundamental issue in vegetation science (Boer and Stafford, 2003; Guisan and Zimmerman, 2000; Korner, 1994; Nicholas et al., 1998; Zhang et al., 2005). Variables such as climate (temperature, rainfall and radiation), soil (slope and texture, nutrients and porosity) and the grazing animal determine presence and abundance of species in a pasture (Boer and Stafford, 2003; Gastó et al., 1993; Korner, 1994; Nicholas et al., 1998).

In the south of Chile 91% of pastures are naturalised and 9% are sown pastures (INE, 2007). The most common naturalised pasture species are *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Leontodon nudicaulis* L., *Hypochaeris radicata* L. and *Taraxacum officinale* L. A large percentage of the naturalised pastures have a degree of degradation, with negative productive, environmental and social consequences (Ellies, 2000). Management practices introduced have been focused on achieving system sustainability, but that has not been completely resolved. The uses of fertiliser, sowing of seeds and grazing management have been applied to restore degraded pastures. As result of these inputs, significant changes have occurred in the botanical composition with a succession from a dominant naturalised vegetation community with low forage value towards a more productive plant community with better forage value (Pacheco, 1997; Siebald et al., 1983).

Species such as *Lolium perenne* L., *Bromus valdivianus* Phil. and *Holcus lanatus* L. dominate in Spring, when light, water and soil nutrients are favorable, while species such as *Agrostis capillaris* L. and flatweeds dominate during summer season (López et al., 2012). High value forage species such as *Lolium perenne* L., have been shown to have the capability to respond positively to soil resources, adjusting their growth according to varying conditions (López et al., 2006). For example, fertilizer combined with controlled grazing stimulates plant-plant competition, increasing *L. perenne* tillering, population and yield over the growth of *Agrostis capillaris* L., and other stress tolerator species (McKay and Lambert, 2011; Flores et al., 2013). Other studies have involved competitive strategies of species such *Bromus valdivianus* and *L. perenne* under soil water restriction. When they were under competition and water restriction, *B. valdivianus* will increase under regular lengthy dry periods and *L. perenne* dominates in the pasture in an adequate soil moisture (López et al., 2013).

The relevance of the above analyses is that each study can determine the effect of some management and environmental variables on different pasture species. The challenge is to determine how all environmental factors, such as climate and management factors (e.g. soil temperature, fertilizer application strategies) influence species abundance in the pasture ecosystem. One approach to determining the relative effect of environmental factors on pastures species is using meta-analysis with a complex data set that contains potential environmental factors and measurements of the abundance of species (Erickson and Nosanchuk, 1992). The integration of this data set in meta-analysis could provide a starting point to define ecosystems traits that should be taken into account to increase the potential sustainability of the system and productivity of the plant community. This approach provides the potential to develop models to predict abundance of species in the pasture and the key factors involved (Zhang et al., 2005).

Some research has focused on the relationships between pasture species and the environment through the identification of the most important variables for plant abundance, for example; soil available potassium, mean annual temperature, mean annual precipitation (Duff et al., 2012), soil Olsen P, soil bulk density, slope and annual P fertiliser (Zhang et al., 2005). Predictive models are able to correlate plant species and specific environment variables affecting the species distribution and abundance (Guisan and Zimmerman, 2000, Zhang et al., 2005). In particular, the use of data mining has become an effective tool in querying and extracting useful information, patterns and trends related to plant population dynamics, community botanical composition and community productivity from large quantities of existing data (Gorunescu, 2011; Liao et al., 2012).

Decision tree is a data mining method that has increased application in environmental modelling for sustainable ecological management of pasture (Corson et al., 2007; Duff et al., 2012; Wan et al., 2009; Zhang et al., 2005). A Decision tree model has been used to predict species abundance (Zhang et al., 2005), pasture production (Zhang et al., 2006), vegetation classification (Yang et al., 2003) and nitrogen utilisation efficiency in pastures (Zhang and Tillman, 2007) showing the ability to predict the relative importance of factors involved in pasture management.

The plant functional groups hypothesis is that plant species that use environmental resources such light, water, nitrogen, in a similar manner or respond in a similar way to disturbances, can be grouped together (Walker, 1992). Such a set of species has common effects in a specific ecosystem stability (Korner, 1994) and are essential to maintain stability (Noble et al., 2007; Walker, 1992). The characteristics used to classify species into functional groups depend on the ecosystem type and objective of the studied objective (Walker, 1995). In humid temperate climates, naturalised pastures have been shown to be able to be studied based on functional groups rather than individual species (Lambert et al., 1986; Nicholas, 1999; Zhang et al., 2005).

In this paper a decision tree modelling approach was used to model the relative abundance of functional groups in a pasture in Valdivia, in the south of Chile. Traditional regression modelling approach was also used to provide a comparison for the performance of the decision tree model. The objectives of this study were to identify the most important variables influencing the abundance of functional groups of species in a naturalised pasture in a humid temperate region in the south of Chile; to develop a decision tree and regression model for the relative abundance of functional groups; and evaluate the performance of the decision tree model prediction with respect to model fit in the validation of data observation.

2. MATERIAL AND METHODS

2.1. Study area and data set

The study was carried out at Santa Rosa Research Station of Universidad Austral de Chile (39º 47' 26" S, 73º 14' 12" W) 9 km east of Valdivia city, Chile. The altitude is 25 m a.s.l., with an average annual rainfall of 2350 mm. The soils are classified as Duric Hapludand soils Valdivia serie (IREN, 1978).

On April 2010, three strategies of pasture improvement were established on a degraded naturalised pasture. The evaluation finished in April 2013. The original pasture (naturalised non fertilised pasture: NFP) was found to be dominated by *Agrostis capillaris* L., *Anthoxanthum odoratum* L. and flatweeds. The pasture improvement strategies were: a) Naturalised fertilised and limed pasture (FP); b) Sown pasture (seed rate: 25 kg ha^{-1} *Lolium perenne* L. cv. Arrow AR1 and 5 kg ha^{-1} *Trifolium repens* L. cv. Huia [Mixed]); c) Sown diverse pasture (seed rate: 9 kg ha^{-1} *L. perenne* cv. Arrow AR1, 5 kg ha^{-1} *T. repens* cv. Huia, 4 kg ha^{-1} *Dactylis glomerata* L. cv. Ella and 27 kg ha^{-1} *Bromus valdivianus* Phil. nativo [Diverse]).

Each pasture occupied 400 m^2 plots, distributed according to a complete randomised block design with three blocks. A low fertility level was measured (Flores et al., 2013), due to low levels of K (68 cmol$^+$kg^{-1}), the total bases exchange (K, Na, Ca and Mg was 70.5 cmol$^+$ kg^{-1}) and a high Al saturation (13%), defined as the exchange Al over the effective cation exchange capacity. The soil showed high level acidity and high P sorption capacity (P available 8.5 mg^{-1} kg^{-1}).

All the sown pastures received the same annual amount of fertiliser and lime as the FP (180 kg N ha^{-1} year^{-1}, 120 kg P ha^{-1} year^{-1}, 120 kg K ha^{-1} year^{-1} and 2 t ha^{-1} year^{-1} of $CaCO_3$ as lime). Each plot was grazed by 25 two years old sheep, Austral breed, live weight 55 ± 2.42 kg (average ± SEM) and body condition 2.71± 0.27 (average ± SEM), from when pastures mass reached an equivalent of 2100 to 2300 kg DM ha^{-1} down to 1000 to 1200 kg DM ha^{-1} (Matthews et al., 2004). If the pasture did not reach the pregrazing mass criteria, the plots were grazed 60 days after the previous grazing event.

Pasture evaluations were conducted when the average of each plot treatment reached the pregrazing criteria. The pasture samples were taken seasonally (Spring, Summer, Autumn and Winter) for three years (April 2010-April 2013), to determine botanical composition by manual separation and the proportion of each species that made up the dry matter basis was calculated. A total of 144 samples were collected using a stratified random method (Erickson and Nosanchuk, 1992). The herbage was cut from a sampling area of 0.1 m^2 at ground level using a trim technique (Radcliffe et al., 1968). The relative abundance of the species was calculated as the percentage of above ground biomass out of the total aboveground biomass in a sample (Table1).

Table1. Botanical composition (%) and pasture production per year (kg DM $ha^{-1}y^{-1}$) during three years of evaluation.

	2011				2012				2013			
Plant species	Mixed	Diverse	NFP	FP	Mixed	Diverse	NFP	FP	Mixed	Diverse	NFP	FP
Lolium perenne L.	69	40	3	3	65	73	0	8	41	46	2	14
Bromus valdivianus L.	19	19	17	5	12	16	5	4	16	4	5	7
Holcus lanatus L.	2	5	15	6	6	4	18	22	6	4	6	3
Agrostis capillaris L.	2	5	6	34	3	1	34	24	9	6	29	9
Anthoxanthum odoratum L.	2	2	3	4	3	0	6	7	2	3	5	9
Dactylis glomerata L.	0	0	6	0	0	0	1	0	1	2	0	2
Festuca arundinacea Schreb.	0	2	1	1	0	0	0	3	3	0	1	3
Trifolium repens L.	5	16	17	1	6	2	10	5	12	13	2	9
Trifolium subterraneum L.	0	0	0	0	0	0	0	0	0	0	0	1
Lotus uliginosus Schkur.	0	0	0	0	0	0	0	1	0	0	1	0
Leontodon nudicaulis L.	2	6	19	25	3	2	22	17	6	5	31	25
Cirsium vulgare (Savi) Ten.	0	0	1	1	0	0	0	1	0	0	2	1
Rumex acetosella L.	0	0	0	0	0	0	0	0	0	0	13	16
Taraxacum officinale Weber.	0	2	11	15	1	0	2	6	4	3	4	2
HFRG	90	67	72	16	84	94	25	37	66	68	14	29
LFRG	3	7	9	38	6	1	41	30	11	9	33	18
legumes	5	17	17	1	6	2	3	6	12	13	3	10
Flatweeds	2	10	33	45	4	3	24	26	11	10	50	43
Productivity (kg DM ha^{-1} y^{-1})	9453	7880	4190	8415	10921	10833	5854	8443	11550	13734	6188	12800

NFP non fertilised pasture; FP Fertilised pasture; HFRG high fertility response grasses; LFRG low fertility tolerance grasses

Pre-grazing and post-grazing herbage mass (kg DM $ha^{-1}y^{-1}$) of each plot was calculated through the relationship between the compressed pasture height and the herbage mass. These data was used to determine annual pasture production between April 2010 and April 2013. The pasture compressed height was measured with a rising plate meter (Earle and McGowan, 1979).

Environmental data collected included pasture management variables (pH, organic matter, mineral nitrogen, Olsen-P, aluminium saturation), soil properties (penetration resistance, mean daily soil water [annual, Spring, Summer, Autumn and Winter], mean daily soil temperature [annual, Spring, Summer, Autumn and Winter]) and climatic variables (rainfall [annual, Spring, Summer, Autumn and Winter]).

There were usually 14 plant species identified in all the plots. Functional groups were classified following the method outlined by Lambert et al., (1983) which defined functional categories (grass, legume and forb) and additionally each category was further classified considering its nutrient response as used by Zhang et al., (2005). Thus, using the same criteria in the present trial there were four functional groups: high fertility response grasses (HFRG) e.g. *Lolium perenne* L., *Bromus valdivianus* Phil., *Holcus lanatus* L. *Dactylis glomerata, Festuca arundinacea*; low fertility response grasses (LFRG) e.g. *Agrostis capillaris* L., *Anthoxanthum odoratum* L.; legumes e.g. *Trifolium repens* L., *Trifolium subterraneum* L., *Lotus uliginosus* Schkur., and flatweeds e.g. *Leontodon nudicaulis* L. Banks ex Lowe, *Hypochaeris radicata* L., *Taraxacum officinale* Weber, *Cirsium vulgare* (Savi) Ten. When soil fertility was low the pasture was dominated by *A. capillaris* L., *A. odoratum* L. and broad leaf weeds and when soil fertility was high the pasture was dominated by *L. perenne* L., *B. valdivianus* Phil., *H. lanatus* L., *T. repens*.

For soil data information, in each sampling plot, 60 soil samples were collected randomly (0-20 cm depth) in order to make twelve composite samples (each sample a composite of 5 subsamples). Whole soil samples were air dried before determining pH, organic matter, soil mineral nitrogen, Olsen P, aluminium saturation following Sadzawka et al., (2006) method. Soil mineral nitrogen was determined as $N–NO_3$ and $N–NH_4$ using KCl as extract and Kjeldahl digestion method (Bremner, 1996). Olsen P was determined by extraction with sodium bicarbonate as $NaHCO_3$ (Olsen, 1954). This information was taken once time per year before fertiliser application.

Soil resistance to penetration was measured in the field, pre-grazing and post-grazing (10 determinations for each plot) using a penetrometer (06.01 Hand Penetrometer, Eijkelkamp, Agrisearch Equipment, Giesbeek, The Netherlands) to characterize the soil mechanical strength in the first 10 cm of soil. Water content and temperature of the soil, time domain reflectometry (TDRs) (3 repetitions, SM200 soil moisture sensors, Delta T Devices, Burwell Cambridge, United Kingdom) and temperature sensors (Pt100 soil temperature sensors, ZIEHL Industrie Elektronik GmbH + Co KG, Stuttgart, Germany) were installed in December 2010 at 10 cm depths in each treatment. The TDR sensors were calibrated following the technique proposed in the SM200 User Manual 1.1 (Delta-T Devices Ltda., 2006). Rainfall was measured at a meteorological station located in the study area (Table 2). The measurements took place every 30 minutes.

Table 2 Variables used in the decision tree and regression analyses

Variable symbol	Units	Range	Variable description
pH	–log 10 [H+]	5.5-6.1	Soil pH
OM	%	13.3-16.6	Soil organic matter
N_mineral	(mg/kg)	28-60.4	Soil mineral nitrogen
Olsen_P	(mg/kg)	8.5-12.7	Soil Olsen P
Al_sat	%	0.4-14.3	Soil aluminum saturation
Pntr	kpa	1190-3081	Soil penetration resistance
Vol_y	%	4-98.8	Annual daily average soil water content
Vol_sp	%	12.5-44.9	Spring daily average soil water content
Vol_su	%	2-41.1	Summer daily average soil water content
Vol_au	%	3.3-68.8	Autumn daily average soil water content
Vol_wi	%	11.4-89.5	Winter daily average soil water content
T_y	°C	8.1-18.3	Annual daily average soil temperature
T_sp	°C	8.6-21.1	Spring daily average soil temperature
T_su	°C	21.1-11.6	Summer daily average soil temperature
T_au	°C	8.1-18.6	Autumn daily average soil temperature
T_win	°C	4.3-12.4	Winter daily average soil temperature
Rain_y	mm	103.5-149.9	Annual rainfall
Rain_sp	mm	30.7-151.4	Spring rainfall
Rain_su	mm	47.1-109.7	Summer rainfall
Rain_au	mm	114.3-164.5	Autumn rainfall
Rain_wi	mm	254.3-312.1	Winter rainfall
Rain_warm	mm	82.7-200.8	Spring and Summer rainfall

2.2 Model development and assessment

Decision tree models and regression models for relative abundance of functional groups were analysed in SAS Enterprise Miner, Version 12.3 (SAS Institute Inc, 2011). The procedure for developing a decision tree model was to split the data of the target variable based on its response to input variables. F test was chosen as the split criterion in developing the decision tree models with a significance level of $P \leq 0.05$ (Fernandez, 2003). Six was the maximum tree level and 11 the minimum observations required for a split search (Fernandez, 2003). In regression analyses, a forward stepwise approach was used to select the variables with a significance level of $P \leq 0.05$ (Erickson and Nosanchuk, 1992).

The dataset used in the development of decision tree and regression models was randomly partitioned in to training data (70% of total) and validation data (30% of total). The decision trees and regression models were built using the training data and fit was assessed using the validation data, which was necessary to achieve a good model output and prevent overtraining (Dunham, 2002). The measure of average squared error (ASE) was used in the data assessment and the best decision tree or regression was selected as that which had the smallest ASE.

The decision tree structure contained prediction of the relative abundance of a functional group from a group of variables defined by input variables and their split point. Split point reflect is the value in which the dependant variable response to independent variable. The first split represented the most important variable which best explained the variance. This method was used to divide in to branches until no further division could be made.

2.3 Evaluation of model prediction

The evaluation of the model prediction was derived using observations from the validation data against decision tree model predictions, using the observation/prediction regression method (OP). This method is strongly recommended by Pineiro et al., (2008) to evaluate models and to test the significance of slope=1 and intercept=0. Coefficient of determination (R^2) was used as measure of the proportion of variance in the observed values that was explained by the predicted values. Root mean square deviations (RMSD) estimates the mean deviation of the predicted values with respect to the observed values and was used to show model performance (Gauch et al., 2003; Lobell et al., 2005; Pineiro et al., 2008).

3. RESULTS

3.1. Decision trees

Figures 1, 2, 3 and 4 display the decision tree models for relative abundance of functional groups HFRG, LFRG, legume and flatweeds, respectively. For HFRG, soil mineral N was the most significant variable influencing relative abundance. Olsen P was the most significant variable influencing LFRG and flatweeds while for legumes soil pH was the most significant variable. Usually, annual, Winter, and Spring daily average soil water contents, Winter rainfall, soil aluminium saturation, Spring daily average soil temperature and soil penetration resistance had secondary roles in influencing abundance of functional groups.

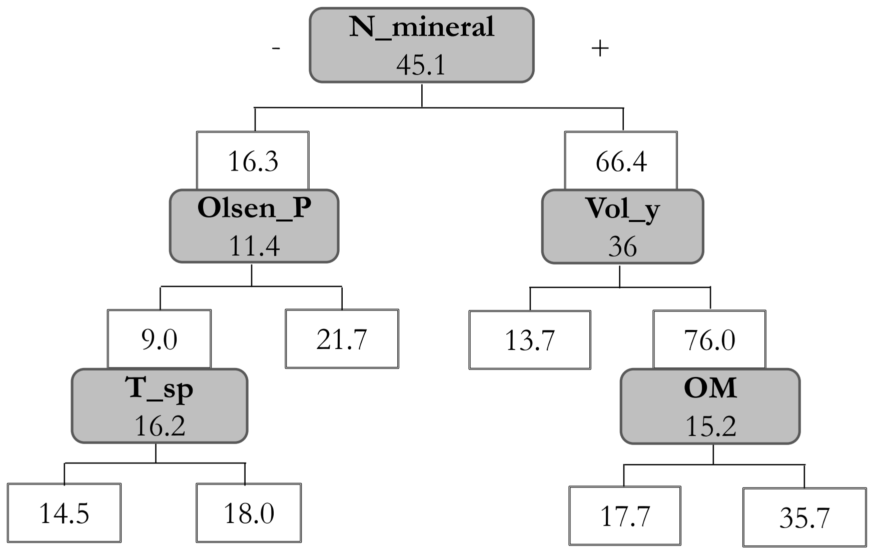

Figure 1. The decision tree model for relative abundance (%) of high fertility response grasses (HFRG) in naturalized pasture. Shaded rectangles contain an input variable and split point. Percentage predicted relative abundances are in unshaded rectangles. Prediction goes to the left-side branch (-) if the splitting variable is less than the split-point, and goes to the right-side branch (+) if the splitting variable is equal to, or more than split point. See Table 1 for variable symbols and unit descriptions.

The most significant variables influencing HFRG were N mineral, Olsen P, annual daily average soil water content, Spring daily average soil temperature and soil organic matter. The lowest abundance (9%) was found in pasture with low N mineral (<45.1 mg/kg) and low Olsen P (<11.4 mg/kg). The highest abundance (76%) was found in pasture with high N mineral (>45.1 mg/kg) and high annual daily average soil water content (>36%) (Fig.1).

The most significant variables influencing LFRG were Olsen P, soil aluminium saturation, Spring daily average soil water content, Winter daily average soil water content and soil penetration resistance. The lowest abundance (3.7%) was found in pasture with low Olsen P (<9.2 mg/kg), high soil aluminium saturation (>7.9 %) and low Winter daily average soil water content (<14.3%). The highest abundance (53.6%) was found in pasture with high Olsen P (>9.2 mg/kg) and high Spring daily average soil water content (>28.1%) (Fig. 2).

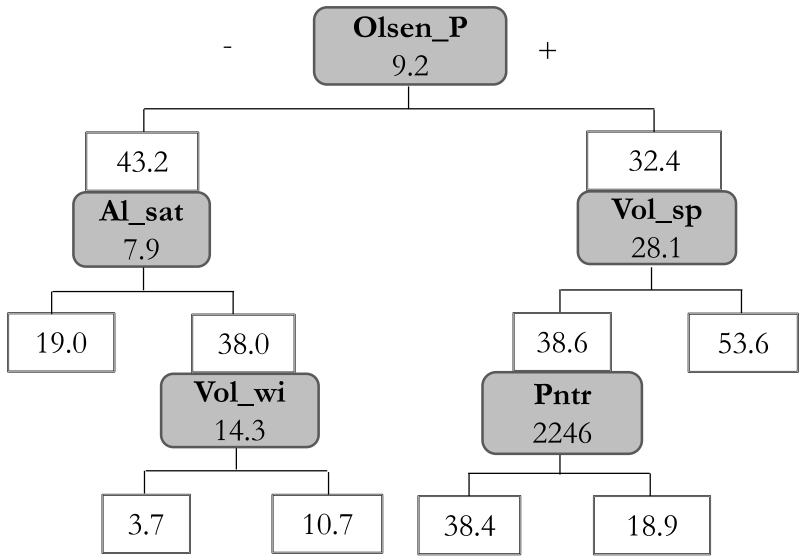

Figure 2. The decision tree model for relative abundance (%) of low fertility response grasses (LFRG) in naturalised pastures. Description and interpretation in caption of Figure 1. See Table 1 for variable symbols and unit descriptions.

Soil pH, Winter rainfall, Spring daily average soil temperature and soil mineral N were the most significant variables influencing the relative abundance of legume. The highest abundance found was 30.4% in pastures with pH equal to, or more than, 5.6, Spring daily average soil temperature >16.2°C and high soil pH (>6). The lowest relative abundance of legume (4.4%) was found in pasture with low soil pH (<5.6) (Fig. 3).

The most significant variables influencing flatweeds were Olsen P, Winter daily average soil water content, soil penetration resistance and soil aluminium saturation. The highest abundance (45.3%) was found in pasture with low Olsen P (<11.5 mg/kg). The lowest abundance (3.1%) was found in pasture with high Olsen P (>11.5 mg/kg), low soil penetration resistance (2205 kpa) and low soil aluminium saturation (1.7 %).

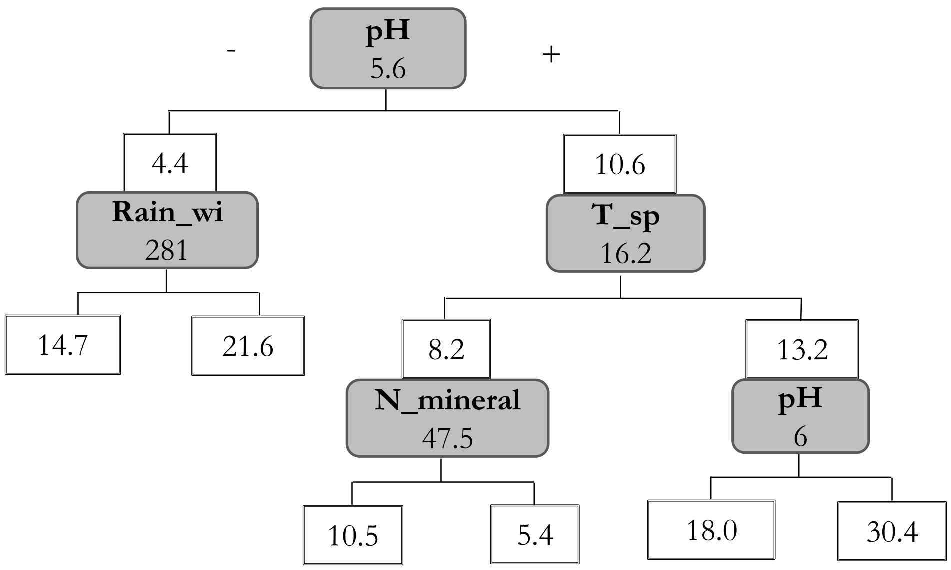

Figure 3. The decision tree model for relative abundance (%) of legume in naturalised pasture. Description and interpretation in caption of Figure 1. See Table 1 for variable symbols and unit descriptions.

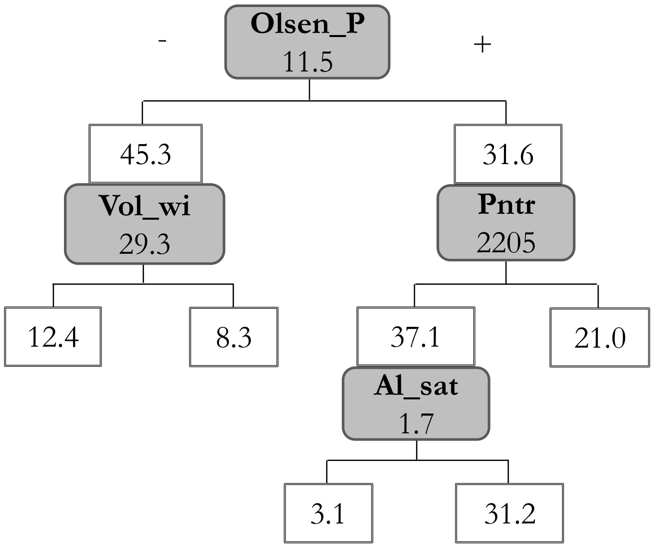

Figure 4. The decision tree model for relative abundance (%) of flatweeds in naturalised pastures. Description and interpretation in caption of Figure 1. See Table 1 for variable symbols and unit descriptions.

Model fit for decision tree and regression models for functional groups are displayed in Table 3. In all functional groups, decision tree had a lower average square error (ASE) than regression models and therefore were better models in predicting the relative abundance of functional groups.

Table 3. Comparison of model fit as indicated by average square error (ASE) between the decision trees and regression models for the relative abundance of functional groups. Root mean square deviation (RMSD) between observed and predicted value for High fertility response grasses (HFRG), Low fertility tolerant grasses (LFRG), legume and flatweed.

Functional group	Average squared error (ASE)		Root mean square deviation (RMSD)
	Decision tree	Regression	
HFRG	12.4	13.4	4.2
LFRG	11.5	12.5	3.8
legume	4.6	5.6	2.7
flatweed	10.4	11.9	3.9

3.2. Evaluation of model prediction

Model predictions were tested using observation validation data and predicted values for relative abundance of functional groups HFRG, LFRG, legume and flatweeds (Fig. 5). There was a good fit between observed and predicted values for relative abundance of functional groups ($P \leq 0.001$) with R^2=0.96 in HFRG, R^2= 0.94 in LFRG, R^2=0.92 in legume and R^2= 0.94 in flatweeds. RMSD between observed and predicted values for relative abundance of species are presented in Table 3.

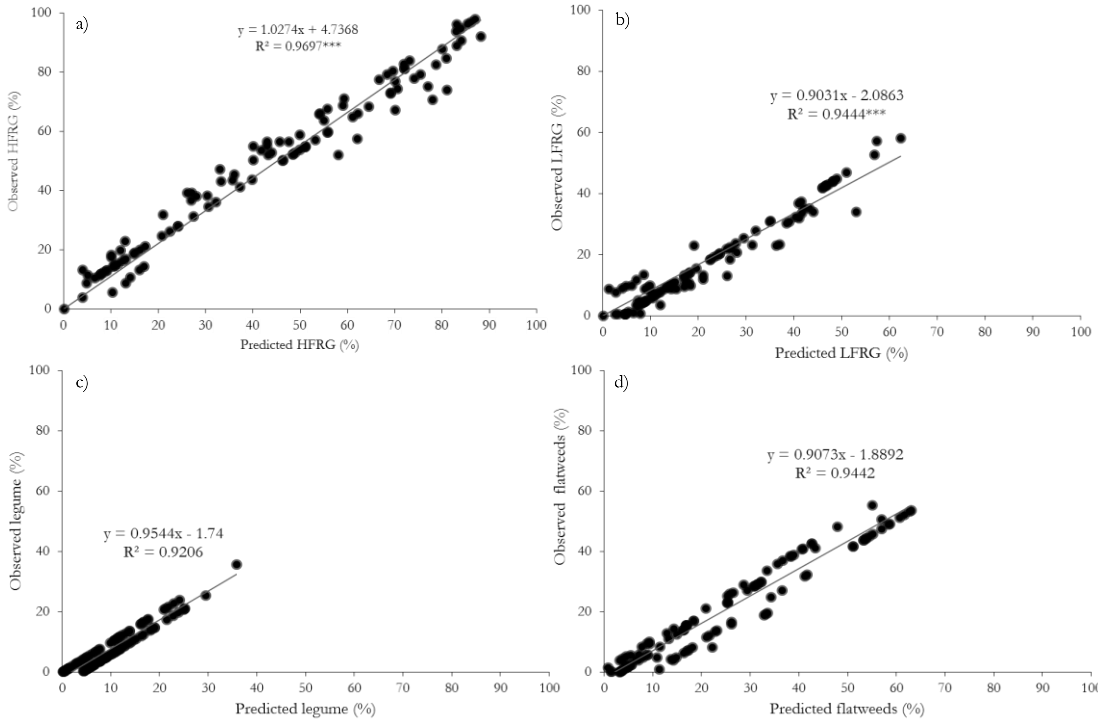

Figure 5. Observation vs. Predicted value regression scatter plots (OP). a) OP relative abundance of high fertility response grasses (HFRG). b) OP relative abundance of low fertility response grasses (LFRG). c) OP relative abundance of legume. d) OP relative abundance of flatweeds. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns, not significant.

4. DISCUSSION

The decision tree models structure revealed the importance of input variables influencing the relative abundance of functional groups in a humid temperate pasture in the south of Chile. The understanding of the variables that are most influential on functional groups on this pasture region was not well known before this analysis.

Variables that can be managed such as soil mineral N, Olsen P and pH influenced species abundance more than uncontrollable environmental variables such as rainfall, which played important but secondary roles in influencing abundance (Wan et al., 2009). Soil mineral N was the most important factor influencing HFRG. With the increased soil mineral N or increased annual daily average soil water content, pasture becomes more HFRG dominant; whereas, the abundance of LFRG and flatweeds was higher when soil Olsen P was low.

N and P fertilizer applications have been shown to produce a possitive effect on grass abundance (Frame, 1991; López et al., 2003; Malhi et al., 2011) and when interacting with available soil moisture increased pasture yields (Stout et al., 1998). Soil water moves nitrogen through the soil solution making it available to be absorbed by the plant (Oulehle et al., 2012). However, when there is a low Olsen P and low soil temperature during Winter and early Spring (Teitzel et al., 1991) relative abundance of HFRG is reduced and LFRG and flatweeds abundance is encouraged.

The decision tree models provide guidelines for fertilizer application in humid temperate pastures. The relationship between N mineral and soil water content located on the HFRG decision tree suggests that the time of N fertiliser application is a determinant factor influencing abundance of HFRG. In this sense, N fertiliser application to pastures in humid temperate pastures should take into consideration the combination of favourable temperature and soil moisture that occurs during early Spring to encourage fast growth of HFRG with increasing N use efficiency. N applied inmediately after the first Autumn rain helps overcome the temporary N deficiency created by soil microbial activity after dry Summer, and encourages pasture growth before it is slowed by cold Winter temperatures (Kemp et al., 1999).

Thus, HFRG being more competitive for soil resouces than LFRG and flatweeds, dominates in the pasture. The N fertiliser application in other times of the year, for example in Summer, may find dry conditions, and in Winter low temperatures that enable other functional groups such LFRG and flatweeds to be relatively more competitive with HFRG.

Olsen P, an indicator of soil P fertility, was the most significant factor affecting relative abundance of LFRG as indicated by the decision tree model. Phosphorus is crucial for the growth of plants and soil microorganisms (Linquist et al., 2011 ; Smith et al., 1999) because maintaining the P status of the soil promotes legume growth and therefore N fixation (Pang et al., 2010). N fixation increases dry matter production and pasture quality (Richardson et al., 2009). Also, P has an indirect effect over Winter on the growth of ryegrass species in the pasture increasing in response to increased soil P (Kemp et al., 1999).

In pastures where soil nutrients are deficient, there is a high percentage of low fertility tolerant species (Grime, 1977). This condition is reflected in the LFRG decision tree with a high predicted value when Olsen P is lower than 11.5 mg/kg. However, increased soil P fertility (for example, through P fertilizer application) encourages LFRG growth when the Spring daily average soil water content increased. The reason behind this may be the competitive ability of species according to the conditions in which species are growing (Grime, 1977). LFRG species commonly have low annual production but with a strong seasonality in spring, due to the favourable environmental conditions in Spring. Increased P fertility will increase LFRG production but will also increase abundance of HFRG species.

The practical usefulness of the LFRG decision tree is that it reflects the characteristics of survival and dominance of LFRG in environments with low levels of phosphorus. Maintaining a high level of P ensures high abundance of HFRG producing a positive effect on the yield and botanical composition of the pasture. A high water soil content increases the P ion in the soil solution, therefore, after rain nutrient absortion by HFRG is facilitated. N application helps the utilization of P through the plant achieving a higher growth rate.

Higher soil pH increased legume abundance. Soil acidity limits species establishment and pasture production, especially legumes (Barrientos et al., 1994). The acidification of the soil is a complex problem related to loss of anions and accumulation of cations. The most important factor affecting the plant growth is aluminium (Zhao et al., 2013) which modifies the cation and ion balance and retards plant growth (Horst et al., 2010; LeNoble et al., 1996). Aluminium solubility depends strongly on pH (Abedi et al., 2013; Mossor-

Pietraszewska, 2001). Normally, Al it is very insoluble, but its solubility is significantly increased under acidic conditions (Ščančar and Milačič, 2006).

To maintain a substantial proportion of legumes in pastures lime application is required to raise soil pH (Haynes and Naidu, 1998). Lime in contact with soil moisture improves soil nutritional efficiency. When the productivity in south of Chile soils is affected by low fertility and high acidity conditions, lime acts by raising the pH of the soil, reducing the saturation of aluminium and substantially improves P uptake by plants (Alfaro et al., 1998). This increase in the pH not only stimulates the presence of legumes, but also of HFRG.

The interaction between species of functional groups can be partially explained by the models. For example, when there is high soil mineral N and Olsen P, HFRG is dominant in the pasture; but LFRG increases when Spring daily average soil water content improves. On the other hand, when Olsen P is low, the competition is codominated by LFRG and flatweeds. Legumes are present when soil pH is higher than 5.6, characteristics of a pasture with high levels of soil nutrients where HFRG are dominant (Marriot et al., 1997, 2009).

The decision trees in this study provide useful guidelines to generate predictions about the performance of pasture species at this site. For example, they can be used as a guide to fertilizer application or management practices to keep the dominance of a specific functional group. Maintaining a threshold of 45.1 mg/kg N with an Olsen P more than, or equal to, 11.4 mg/kg will result in HFRG dominating the pasture. Less than 11.4 mg/kg soil Olsen P, LFRG abundance begins to be superior to that of HFRG (Wan et al., 2009). Flatweeds, meanwhile are more abundant when Olsen P is less than 11.4 mg/kg, coinciding with decline of HFRG and the increase of LFRG in the pasture.

The Decision tree model performed better than the regression models with respect to model fit and also provided insight if the input variables chosen are properly significant. Similarly, an application of the decision tree in predicting species abundance in New Zealand pastures (Wan et al., 2009) showed that the decision tree model performed significantly better than regression models. Decision tree models are also being used succesfully with remote sensed data (Yang et al., 2003) and in precision agriculture (Waheed et al., 2006). The overall evaluation model prediction had a high correlation between the predicted value by decision tree model and the observed value. The high R^2 found is considered an assesment of model performance (Pineiro et al., 2008; Smith and Rose, 1995). In other words, the decision tree models for HFRG, LFRG, legumes and flatweeds were effective in predicting the relative abundance of the functional groups.

These results suggest that the decision tree model can be used as a tool to predict the abundance of functional groups and to discover the principal key factors influencing their abundance. Its use should be connected with environmental and management conditions to obtain an increase or decrease in a specific functional group of species. In this sense, it could determine and predict the consequences when fertiliser and lime are applied, the impact of a summer drought and of low temperatures. The successful integration of the most important variables influencing abundance of species with the decision tree models provided a new approach to understand the relative abundance of functional groups in humid temperate pastures in the south of Chile and the potential impacts of the development of practical management of these pastures.

5. CONCLUSIONS

The decision tree models developed in this study revealed management variables are the main factors influencing abundance of functional groups in a humid temperate pasture in the south of Chile. Rainfall had secondary roles compared with soil and management variables. Olsen P was the most important variable influencing LFRG and flatweed abundance, while soil mineral N, and pH were the most important for HFRG and legumes, respectively. The decision tree models also had better performance than regression models for predicting relative abundance of functional groups with respect to model fit. Evaluation of the decision tree model prediction showed a good fit between observed and predicted values for HFRG, LFRG, legumes and flatweeds with a high predictive ability.

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Chapter 5

Predictive modelling of the main determinants of annual and seasonal pasture productivity in the south of Chile

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ABSTRACT

We studied the environmental and management variables influencing annual and seasonal pasture productivity using decision tree models to predict annual and seasonal pasture productivity. Data for developing the decision tree models were collected at the Santa Rosa Research Station of Universidad Austral de Chile, Valdivia city, Chile, from April 2010 until April 2013. Evaluation of model prediction used the observation/prediction regression method (OP). Results showed that the soil mineral N (47.5 mg/kg) was the most important variable influencing the annual pasture productivity of 13786 kg/ha, while pH, rain and soil aluminum saturation were the most important for Summer, Autumn and Winter pasture productivity, respectively. The high spring productivity found in pastures (4746 kg/ha) was observed with Spring rainfall equal or more than 91.3 mm. The highest Summer productivity (2712 kg/ha) was found in pasture with a pH more than 5.6 and high N mineral (>50.8). Autumn productivity when the pasture had an Autumn rainfall more than 131.4 mm, was 3172 kg/ha and the highest Winter productivity (3012 kg/ha) was found in pasture with low soil aluminum saturation and high Winter rainfall. The decision tree model showed a high correlation between the predicted value of the model and the observed value. The positive performance of the decision tree models implies this methodology can be used to predict pasture productivity and to provide practical highlights into relative importance of strategic inputs such as lime and N fertilizer to improve pasture production in humid temperate regions, such as the south of Chile.

Keywords: decision tree, modelling, predicted value, pasture productivity

1. INTRODUCTION

In recent years, considerable research has been conducted in temperate regions, such as the south of Chile, on improved naturalized pastures (Balocchi & López 2009; López et al., 2009; Keim et al., 2014). In these regions, dairy, beef and sheep production are based on grazing systems centered on pastures (Balocchi, 2002) because it is the lowest cost feed (Romero, 2004). Profitability of these animal production systems is affected by annual herbage mass production, seasonal pasture growth and the cost and response to inputs used to increase pasture production. Nitrogen (N) and phosphorus (P) are main growth limiting nutrients (Redel et al., 2007), but when soils are of volcanic ashes origin, such as the Duric Hapludand of the south of Chile (IREN et al., 1978), they are also acidic with high aluminium saturation.

When permanent pastures are in a degraded condition in southern Chile, its annual herbage mass is about 4 t DM ha^{-1} $year^{-1}$, however, this pasture can reach more than 8 t DM ha^{-1} $year^{-1}$ in the second year of fertiliser and lime application (López et al., 2012).

The effects of fertiliser and lime application at supporting high pasture production and their interaction with climate and management have been recognized in southern Chile as well as in pastures of other temperate regions (Keim et al., 2014). The management of soil nutrient availability and climate variability through the year have become on important components of pasture management.

To achieve the maximum pasture potential, is necessary a good decision support management to increase the efficient use of the pasture and to reduce the impact of climate variability (Zhang et al., 2009). The modelling of pasture productivity has been used as a decision support tool with the ability to predict pasture production as response to fertiliser addition. The model correlated pasture productivity with variables such as soil nutrients, topographical features and climate data for New Zealand hill pastures and demonstrated high predictive value (Zhang et al 2006).

There has been an increased interest in environmental modelling for sustainable ecological management of pastures (Corson et al., 2007; Duff et al., 2011; Wang et al., 2009). Examples of predictive models based on decision tree models include: prediction of annual pasture productivity in New Zealand (Zhang et al., 2004), prediction of nitrogen utilisation efficiency in New Zealand pastures (Zhang and Tillman, 2007), and data mining to create a model that determines species response to nutrient availability and that differentiates between functional groups (Zhang et al., 2005).

Data generated from pasture research provided a starting point to develop models for pasture production (Zhang et al., 2006). The estimation of available pasture to feed animals, as well as pasture response to fertiliser application, would be of considerable value to pasture management.

Decision tree modelling was used to predict highly heterogeneous pastures measurements of pasture growth collected from several trials (Zhang et al., 2005). In southern of Chile, there is a large number of evaluations performed on pasture growth related to fertiliser addition and environment variables which could be used to predict pasture response to fertiliser and increase resource efficiency. The predictions take in to account the changes in pasture dynamics and then may be used to modify practices to improve pasture management.

The objectives in this study were to develop a decision tree for seasonal and annual pasture production; to identify the key variables influencing annual and seasonal pasture productivity; and to evaluate the performance of the decision tree model to predict pasture productivity using the observation/prediction regression method (OP).

2. MATERIAL AND METHODS

2.1 Study area

The pasture data were collected at the Santa Rosa Research Station of Universidad Austral de Chile (39º 47' 26" S, 73º 14' 12" W) 9 km east of Valdivia city, Chile, at 25 m a.s.l., with 2350 mm of average annual rainfall. The soils are classified as Duric Hapludand (IREN et al., 1978). On April 2010, three methods of pasture improvement were established on a degraded naturalised pasture. The study was finished in April 2013. There were four treatments: a) Naturalised fertilised and limed pasture (FP) composed by *Agrostis capillaris* L., *Leontodon nudicaulis* L. Banks ex Lowe, *Lolium perenne* L. and *Trifolium repens* L.; b) *L. perenne* and *Trifolium repens* L. sown mixture pasture; c) Sown diverse pasture comprised of *L. perenne*, *T. repens*, *Holcus lanatus* L., *Dactylis glomerata* L. and *Bromus valdivianus* Phil.; and d) Naturalised pasture without lime or fertiliser addition (NFP). Each pasture occupied 400 m^2 plots, distributed according to a complete randomised block design with three blocks.

All the sown pastures received the same annual amount of fertiliser and lime as the NFP: 180 kg N ha^{-1} $year^{-1}$, 120 kg P_2O_5 ha^{-1} $year^{-1}$, 120 kg K_2O ha^{-1} $year^{-1}$ and 2 t ha^{-1} $year^{-1}$ of lime. Each plot was grazed by 25 two years old sheep, Austral breed, live weight 55 ± 2.4 kg (average ± SEM) and body condition 2.7± 0.27 (average ± SEM). Pregrazing herbage mass target was about 2.100 to 2.300 kg DM ha^{-1}. Postgrazing residual herbage mass target was about 1.000 to 1.200 kg DM ha^{-1} (Matthews et al., 2004). The maximum length of defoliation interval was 60 days.

2.2 Dataset

Environmental data included soil pH in both water (1:2.5) and $CaCl_2$, soil organic matter, mineral nitrogen, Olsen-P, aluminium saturation, soil penetration resistance and climatic variables (annual and seasonal rainfall). Sensors placed at 10 cm of soil depth registered annual and seasonal daily average available soil water and mean daily soil temperature.

Pasture productivity was assessed using the rising plate method (Earle & McGowan, 1979) with a calibrated equation: y=174.76x + 232.04 (R^2 0.8). The trim technique (Radcliffe et al., 1968) was used with a 0.1 m^2 quadrat and cutting the pasture to a soil level.

Soil fertility level was determined in each plot. Soil samples were taken from the plots for chemical analysis (0-20 cm depth), such that five soil subsamples comprised one plot sample. Soil samples were air dried and analysed for pH, organic matter, soil mineral nitrogen, Olsen P, aluminium saturation following Sadzawka et al., (2006) method. Soil mineral nitrogen was measured as $N–NO_3$ and $N–NH_4$ using KCl as extract and Kjeldahl digestion method (Bremner et al., 1996).

Olsen P was determined by extraction with sodium bicarbonate (Olsen, 1954). Soil resistance in the field was evaluated with a penetrometer (06.01 Hand Penetrometer, Eijkelkamp, Agrisearch Equipment, Giesbeek, The Netherlands). Thus, penetrometer measurements of the first 10 cm of soil depth were taken in each plot and the soil mechanical strength determined for each case. Soil water content and temperature were measured (3 repetitions per plot) with a TDRs (SM200 soil moisture sensors, Delta T Devices, Burwell Cambridge, United Kingdom and Pt100 soil temperature sensors, ZIEHL Industrie Elektronik GmbH + Co KG, Stuttgart, Germany). The soil water and temperature sensors were installed in December 2010 at 10 cm depth in each treatment. The TDR sensors were calibrated following the technique in the SM200 User Manual 1.1 (Delta-T Devices Ltda., 2006). Weather variables, such as rainfall, were measured at a meteorological station located in the study area (Table 1). There were a total of 26 variables, which included 21 input variables (independent variables) and 5 target variables (dependent variables; Table 1).

Table 1. Input variables used in the decision tree and regression analyses

Variable symbol	Unit	Range	Variable description
Input variables			
pH	–log 10 [H+]	5.5-6.1	Soil pH
OM	%	13.3-16.6	Soil organic matter
N_mineral	(mg kg^{-1})	28-60.4	Soil mineral nitrogen
Olsen_P	(mg kg^{-1})	8.5-12.7	Soil Olsen P
Al_sat	%	0.4-14.3	Soil aluminum saturation
Pntr	kPa	1190-3081	Soil penetration resistance
Vol_y	%	4-98.8	Annual daily average soil water content
Vol_sp	%	12.5-44.9	Spring daily average soil water content
Vol_su	%	2-41.1	Summer daily average soil water content
Vol_au	%	3.3-68.8	Autumn daily average soil water content
Vol_wi	%	11.4-89.5	Winter daily average soil water content
T_y	°C	8.1-18.3	Annual daily average soil temperature
T_sp	°C	8.6-21.1	Spring daily average soil temperature
T_su	°C	11.6-22.1	Summer daily average soil temperature
T_au	°C	8.1-18.6	Autumn daily average soil temperature
T_win	°C	4.3-12.4	Winter daily average soil temperature
Rain_y	mm	103.5-149.9	Annual rainfall
Rain_sp	mm	30.7-151.4	Spring rainfall
Rain_au	mm	114.3-164.5	Autumn rainfall
Rain_wi	mm	254.3-312.1	Winter rainfall
Rain_warm	mm	82.7-200.8	Spring and Summer rainfall
Target variables			
Annual productivity	kg DM ha^{-1} y^{-1}	5599-14623	Annual aboveground dry matter per hectare
Spring productivity	kg DM ha^{-1} $season^{-1}$	853-8846	Spring aboveground dry matter per hectare
Summer productivity	kg DM ha^{-1} $season^{-1}$	563-3245	Summer aboveground dry matter per hectare
Autumn productivity	kg DM ha^{-1} $season^{-1}$	661-6890	Autumn aboveground dry matter per hectare
Winter productivity	kg DM ha^{-1} $season^{-1}$	872-4948	Winter aboveground dry matter per hectare

2.3 Model development and assessment

Decision tree models and regression models for annual and seasonal pasture production were analysed in SAS Enterprise Miner, Version 12.3 (SAS Institute Inc, 2011). The procedure for developing a decision tree model was to split the data of the target variable based on its response to input variables. The F test was used as split criterion to develop the decision tree models with a significance level of $P \leq 0.05$ (Fernandez, 2003). In regression analyses, a forward stepwise approach was used to select the variables with a significance level of $P \leq 0.05$ (Erickson and Nosanchuk, 1992). Six was the maximum tree level and 11 the minimum observations required for a split search (Fernandez, 2003).

Model assessment is an important step towards developing a decision tree, as it enables a good model output, prevents overtraining and fits only trained data (Dunham, 2006). The average squared error (ASE) was used to assess the model. The whole dataset used in the development of decision tree and regression models, was randomly partitioned into two parts: training data (70% of total) and validation data (30% of total). The decision trees and regression models were derived using the training data and fit was assessed using the validation data. The best decision tree or regression was selected as that which had the smallest ASE.

2.4 Evaluation of model prediction

Model predictions were tested using the same data used in their construction as an evaluation of the calibration procedure. The evaluation of model predictions was derived using the observation of validation data against the prediction of the decision tree models, using the observation/prediction regression method (OP). This method is strongly recommended by Pineiro et al. (2008) to evaluate models and to test the significance of slope=1 and intercept=0. The slope=1 and intercept=0 not overestimate the observed data at low values and underestimate at high values (Pineiro et al., 2008). Coefficient of determination (R^2) was used to indicate how much of the linear variation of observed values was explained by the variation of predicted values (Smith and Rose, 1995). Root means square deviations (RMSD) estimated the mean deviation of predicted value with respect to the observed value and was used to show model performance (Lobell et al., 2005; Pineiro et al., 2008).

3. RESULTS

3.1 Decision trees models

The decision tree models for pasture production in annual and seasonal bases are presented in Figures 1, 2, 3, 4 and 5. The decision tree model for annual pasture productivity (Figure 1) predicted that when soil mineral N was more than 47.5 mg kg^{-1} and warm rainfall equal to or more than 199.7 mm, the predicted annual productivity was 13786 kg DM ha^{-1}y^{-1}, which was the highest annual production. When the total Spring and Summer rainfall was less than 199.7 mm the Spring daily average soil temperature was more than 17.3°C, and the Spring daily average soil water content equal or more than 36.1 %, the predicted value for annual productivity was 10971 kg DM ha^{-1}y^{-1}.

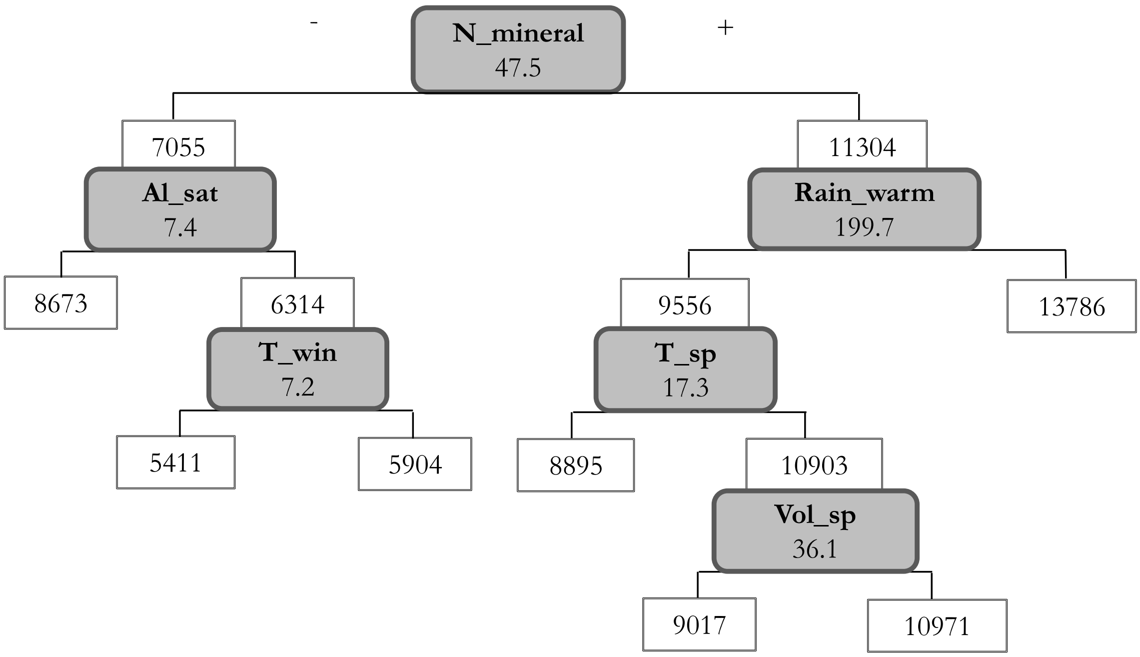

Fig. 1 The decision tree model for annual productivity. Shaded rectangles contain an input variable and split point. Predicted productivity (kg DM ha^{-1}y^{-1}) is in unshaded rectangles. Prediction goes to the left-side branch (-) if the splitting variable is less than the split-point, and goes to the right-side branch (+) if the splitting variable is equal to, or more than split point. See Table 1 for variable symbols and unit descriptions.

The lowest annual pasture production (5411 kg DM $ha^{-1}y^{-1}$) was found when the soil N mineral content was low (<47.5 mg kg^{-1}), the soil aluminium saturation was high (7.4 %) and the Winter daily average soil temperature was less than 7.2 °C (Figure 1).

Soil mineral N, Spring rainfall, pH, soil penetration resistance and Spring daily average soil temperature were the most significant variables influencing Spring pasture productivity. The greatest Spring productivity was 4746 kg DM $ha^{-1}season^{-1}$ found in pastures with Spring rainfall greater than 91.3 mm. The lowest Spring productivity (1161 kg DM $ha^{-1}season^{-1}$) was observed when soil mineral N was less than 31.7 mg kg^{-1}, with a low pH (<5.6) and soil penetration resistance more than 1648 kpa (Figure 2).

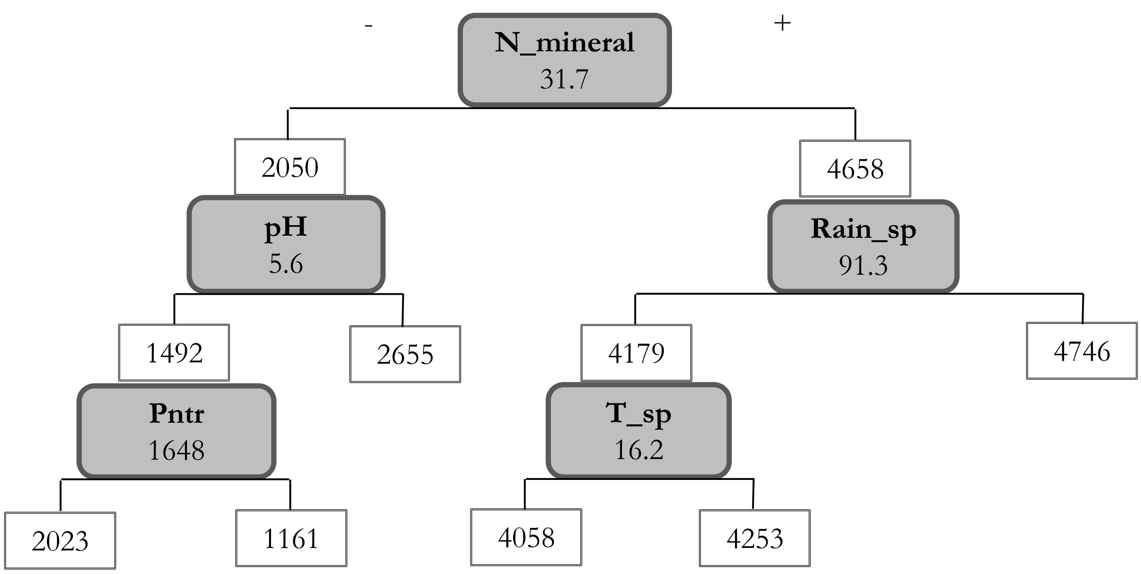

Fig. 2 The decision tree model for spring productivity (kg DM $ha^{-1}season^{-1}$). See the caption of Fig.1 for the description of decision tree model interpretation.

Pasture Summer production was strongly determined by pH, soil mineral N and Olsen P, Summer daily average soil and temperature, Spring and Summer rainfall and

Summer daily average soil water content. The greatest pasture Summer production (2712 kg DM $ha^{-1}season^{-1}$) was observed when the soil had a pH greater than 5.6 and levels of soil mineral N larger than 50.8. The lowest Summer production (1257 kg DM $ha^{-1}season^{-1}$) was associated to soil pH lower than 5.6, with low Summer daily average soil temperature minor than 19.7°C and soil Olsen P less than 12.3 mg kg^{-1} (Figure 3).

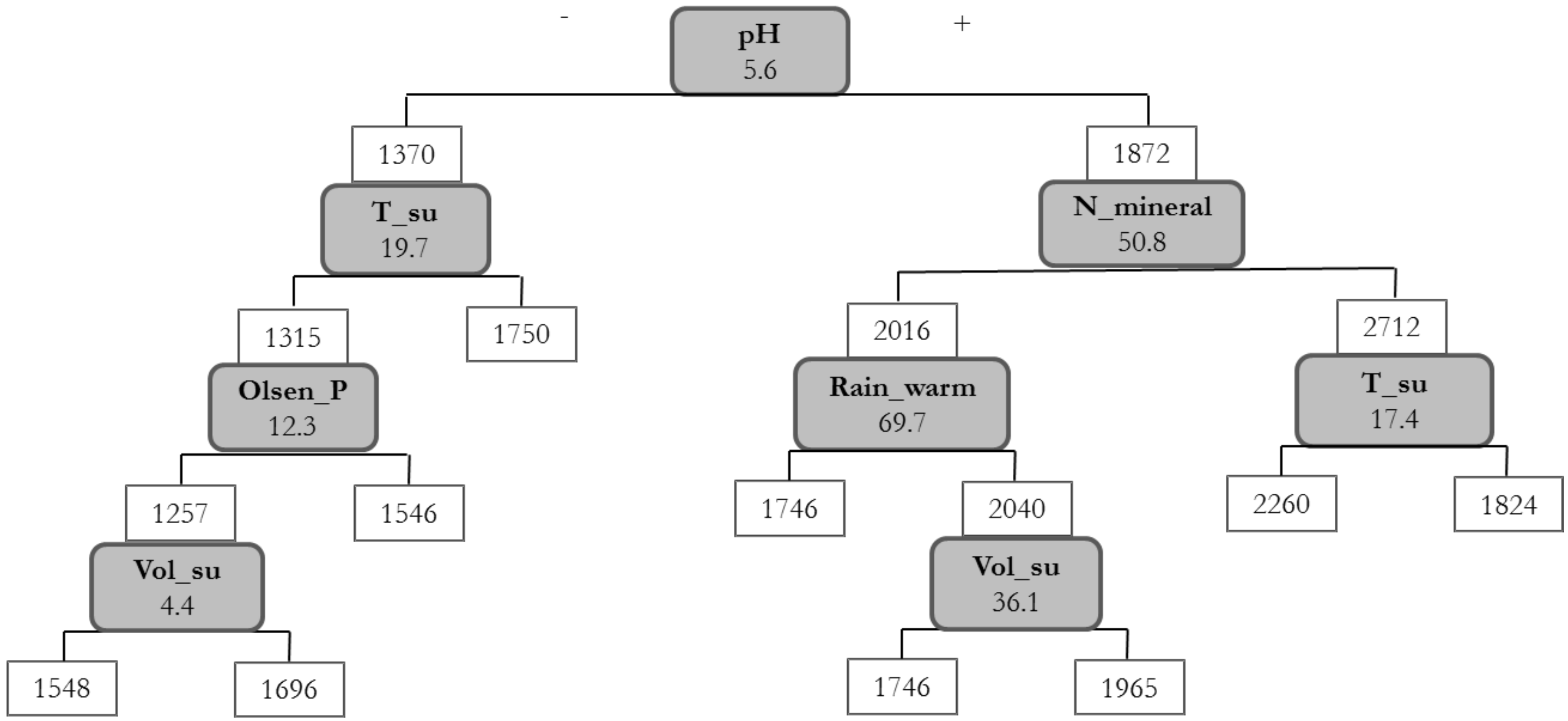

Fig. 3 The decision tree model for summer productivity (kg DM $ha^{-1}season^{-1}$). See the caption of Fig.1 for the description of decision tree model interpretation

Pasture production during Autumn was strongly determined by the rainfall levels, Summer mean daily soil temperature, soil aluminium saturation, pH and Olsen P, Autumn daily average soil temperature and soil penetration resistance (Figure 4). The lowest Autumn pasture production (1322 kg DM $ha^{-1}season^{-1}$) was observed with low Autumn rainfall (<131.4 mm) and high soil aluminium saturation (>7.4 mg kg^{-1}) (Fig.4). When the Autumn rainfall was greater than 131.4 mm, the predicted Autumn pasture production was 3172 kg DM $ha^{-1}season^{-1}$, which constituted the greatest pasture production observed for this season.

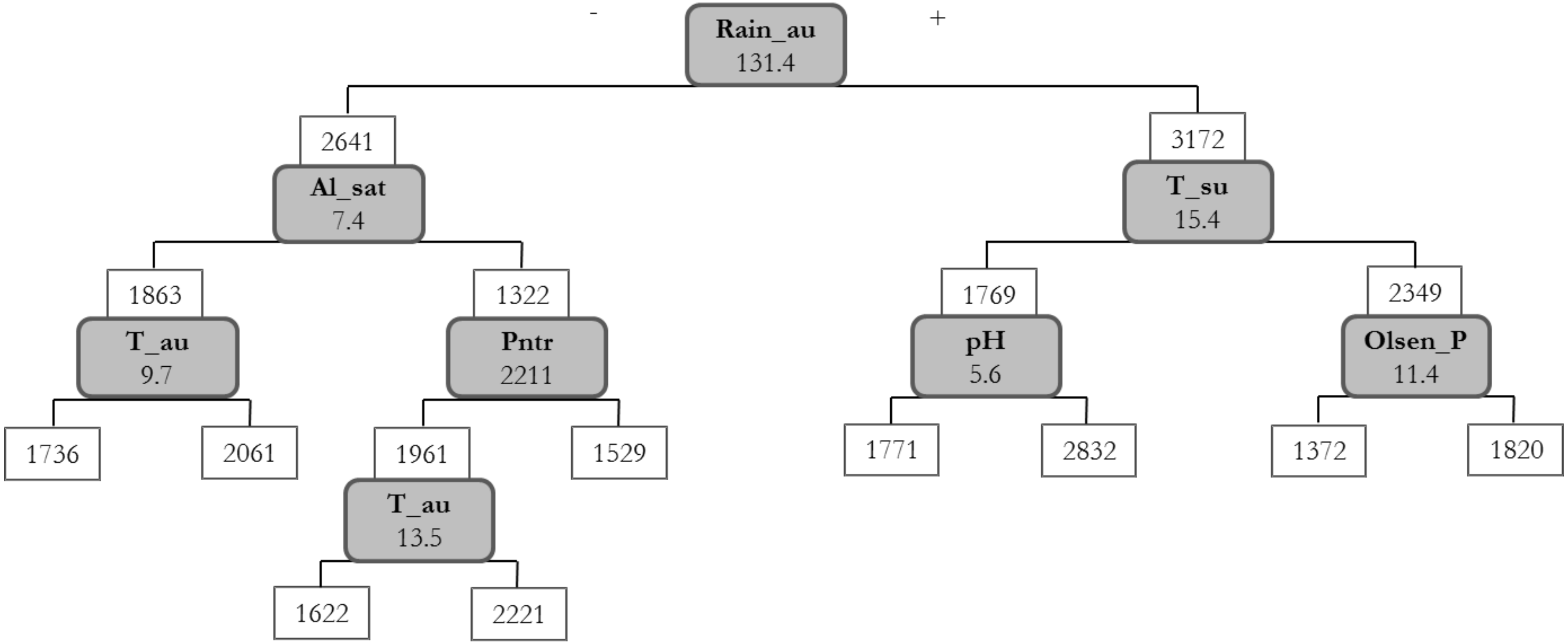

Fig. 4 The decision tree model for autumn productivity (kg DM $ha^{-1}season^{-1}$). See the caption of Fig.1 for the description of decision tree model interpretation

Variables determining pasture winter production were soil aluminium saturation, Winter rainfall, Winter daily average soil temperature, soil mineral N and soil penetration resistance. The highest Winter productivity (3012 kg DM $ha^{-1}season^{-1}$) was measured in pasture growing in low soil aluminium saturation and high Winter rainfall. The lowest pasture production was associated to high soil aluminium saturation (>7.4 mg kg^{-1}) (Figure 5).

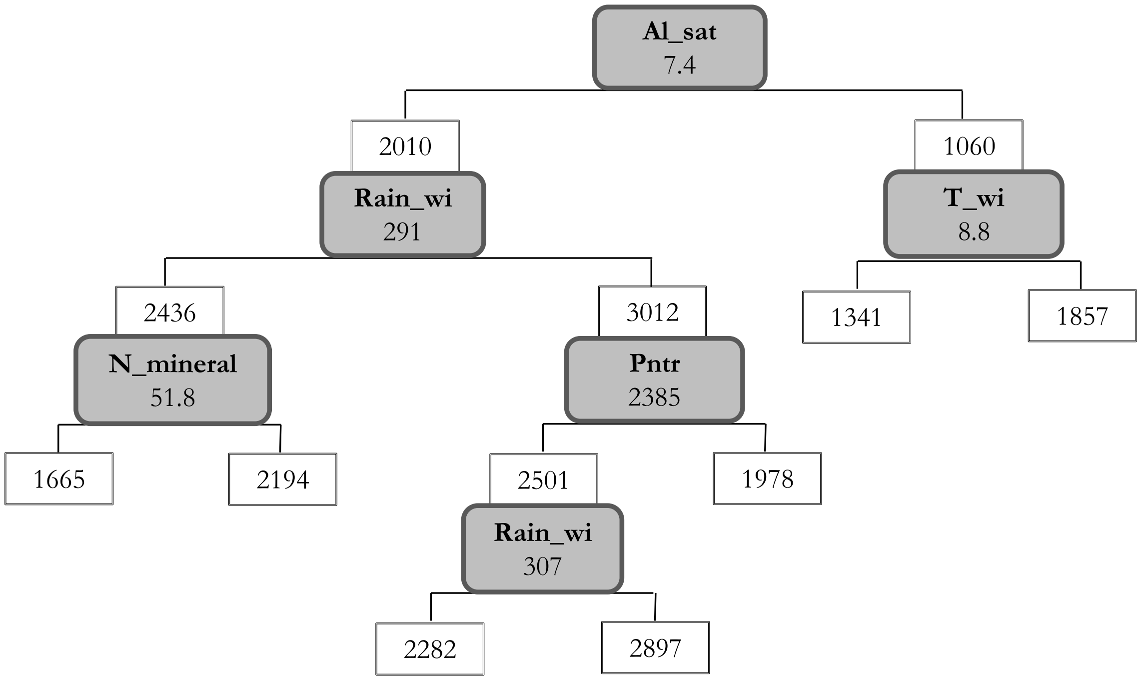

Fig. 5 The decision tree model for winter productivity. See the caption of Fig.1 for the description of decision tree model interpretation.

A summary of model fit for the decision tree and regression models for the annual and seasonal productivities is displayed in Table 2. For annual and seasonal pasture production, the decision tree had a lower average square error (ASE) than regression models and, therefore, were better models for predicting annual and seasonal production.

Table 2. Comparison of model fit as indicated by average square error (ASE) between the decision trees and regression models for annual and seasonal productivity.

Productivity	Variables	Average squared error (ASE)		
		Decisión tree	Regression	p-value
Annual	Input variables	126	209	0.001
Spring	pH, OM, N_mineral, Olsen_P, Pntr, Vol_sp, T_sp, Rain_sp	43	51	0.004
Summer	pH, OM, N_mineral, Olsen_P, Pntr, Vol_su, T_su, Rain_su, Rain_warm	48	53	0.008
Autumn	pH, OM, N_mineral, Olsen_P, Pntr, Vol_au, T_au, T_su, Rain_au	34	39	0.008
Winter	pH, OM, N_mineral, Olsen_P, Pntr, Vol_wi, T_wi, T_wi, Rain_wi	38	45	0.004

3.2. Evaluation of model prediction

Model predictions were tested using validation data and predicted value for annual and seasonal productivity (Fig 6-7).

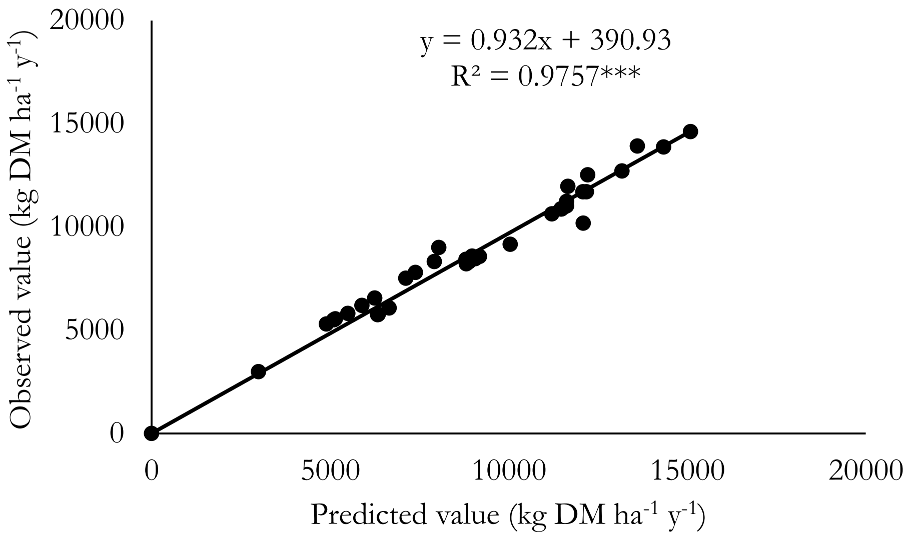

Fig. 6 Observation vs. Predicted value regression scatter plot (OP) of annual productivity. ***$P \leq 0.001$; ns, non significant.

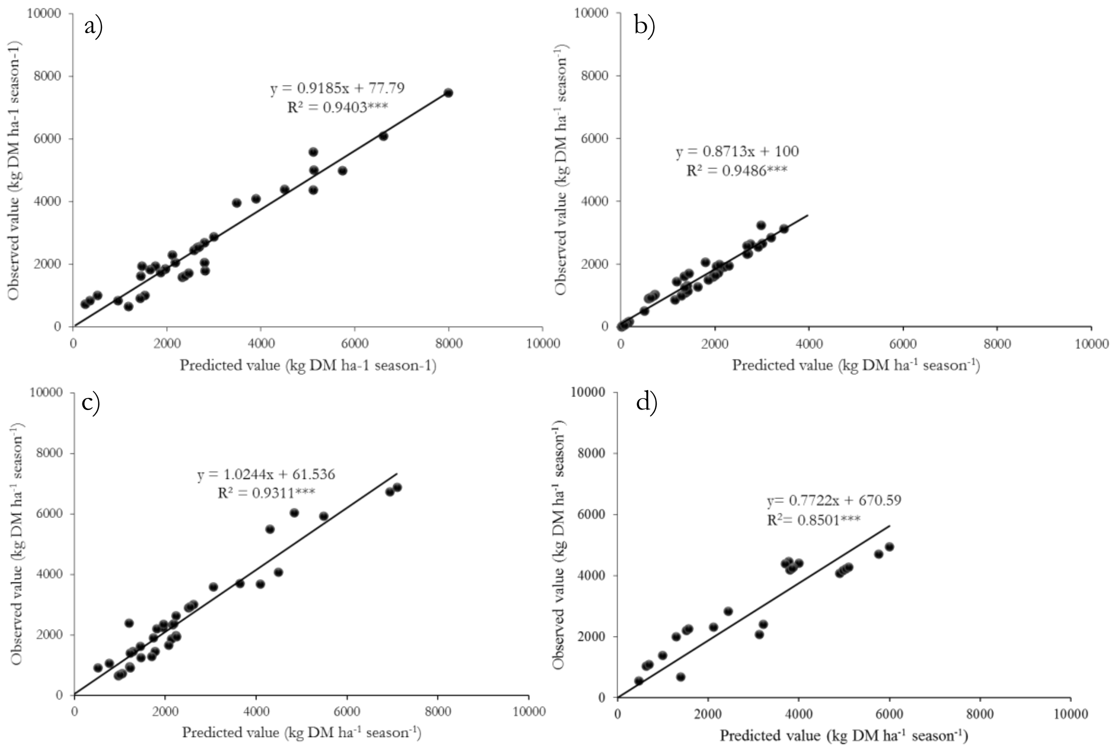

Fig. 7 Observation vs. Predicted value regression scatter plots (OP) for seasonal productivity a) OP for Spring productivity b) OP for Summer productivity c) OP for Autumn productivity d) OP for Winter productivity .$*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; ns, non significant.

Root means square deviations, between observed and predicted values for pasture production were 23.9 (annual), 10.3 (Spring), 19.4 (Summer), 17.5 (Autumn) and 16.8 (Winter). The R^2 between linear variation of observed and predicted values for annual, Spring, Summer, Autumn and Winter productivity were 0.97, 0.94, 0.89, 0.93 and 0.85, respectively.

4. DISCUSSION

The decision tree models obtained were able to predict in a good manner pasture production. The models had a high correlation between the predicted values and the observed values indicating a high predictive accuracy, which was better than the regression models (Table 2, Figure 6-7). Similarly to Zhang et al. (2006), the decision tree models were predictive of the effect of environmental variables on annual and seasonal pasture production revealing the relative importance of environmental and management variables and their interaction.

The models showed that N, pH and aluminium saturation importantly determine annual and seasonal pastures production. These three variables are related to pasture management. Nitrogen stimulates plant establishment and growth as has been positively correlated to pasture herbage mass production (Malhi et al., 2011) especially of fast growing species such as *Lolium perenne* (Tharmaraj et al., 2008).

The dynamics of soil nutrients as N and P and also the organic matter/carbon represent major soil quality indicators (Assefa & Keulen, 2005). These aspects of sustainability can be assessed by examining the consequences of current management regimes on soil nutrients dynamics (Assefa & Keulen, 2005).

Acidic soils without applications of lime tend to increase their acidity with time and nutrients availability decreases (Bronick, 2005). Under acid soil conditions, aluminium nonphytotoxic forms solubilize at low pH, and the toxic ion (Al^{3+}) is released into the soil solution (Brunner & Sperisen, 2013). This form of Al is capable to inhibit root growth and damaging cells at the root apex, which is the most sensitive part of the root to Al^{3+}. Acid soils characteristically contain high amounts of Al^{3+} and low amounts of the base cations such as Ca^{2+}, Mg^{2+} and K^{+}, which are important plants nutrients. Since Al^{3+} and base cations interact at the plasma membrane surface, also determines the plant responses to Al^{3+} exposure (Brunner et al., 2004).

Many plant species are susceptible in acids soils, and their growth and yield are limited by high Al^{3+} conditions. However, native plants communities can tolerate acid soil conditions over large areas. For example, in a temporal comparison in a period of almost 70 years in unfertilised acidic grasslands (2-4 pH) located in a continental climate of Northwestern and Central Europe, *Agrostis capillaris* increased 60% to 87%, *Festuca rubra* increased 25% to 85%, *Holcus lanatus* increased 7% to 28% and *Anthoxanthum odoratum* 49% to 51% (Dupre et al., 2010). In unfertilised naturalised pastures located in temperate climate (5.6 pH), in a time of 3 years, *A. capillaris* increased more than 100% (Chapter 1). On the contrary, when soil pH increased to 6.1, *A. capillaris* may decrease 60% *L. perenne* increased 87% (Chapter 1).

Acidic soils include also toxic levels of manganese (Mn^{2+}) and iron (Fe^{2+}), as well as deficiencies of several essential mineral elements, such as P that constitutes a major limiting factor for plant growth in acidic soils (Kochian et al., 2004). Some species can survive under acidic conditions through mechanisms of tolerance to Al. However, there has been considerable speculation about a number of different Al tolerance mechanisms and it is likely that multiple Al tolerance mechanisms are employed by different plant species (Matsumoto, 2000; Kochian et al., 2005).

Nitrogen is a key mineral nutrient in pasture systems. In temperate grasslands, perennial legumes are very important for the biological N fixation (Nyfeler et al., 2011) that would otherwise need to be added with N fertiliser. The N fixed by the plants is related with the symbiosis legume-rhizobia bacteria (Herridge et al., 2008). Some legume species require infection by a specific *Rhizobium* to create an effective N fixing symbiosis. For example, the symbiosis relationships containing *Rhizobium trifolii* bacteria are well stablished for *Trifolium repens*, *T. subterraneum* and *T. pratense* (Gibson et al., 1975; Brockwell et al., 1982; Chanway et al., 1989).

A summary of a large number of studies investigating N fixation in three perennial forage legumes primarily relating to ungrazed northern temperate/boreal areas, reported rates of N fixation in a range of up to 545 kg N ha^{-1} in *T. repens*, 373 kg N ha^{-1} in *T. pratense* and 350 kg N ha^{-1} in *Medicago sativa* and the values were significantly correlated to legume dry matter (Carlsson & Huss-Danell, 2003).

Rainfall has been recognised as a key factor stimulating pasture production (Zhang et al., 2006), which was an important factor for Autumn pasture growth. In Autumn when rainfall was equal or more than 131 mm could be possible achieve 3172 kg DM ha^{-1}. In Autumn the range of daily average soil temperature (8.1-18.6°C) and soil moisture (3.3-68.8%) allows to achieve a range of pasture growth rate between 661-6890 kg DM ha^{-1}, because tiller regrowth are in recovery after a Summer drought period (Tomlinson and O'Connor, 2004). The N fertiliser application can accelerate the tillering process in these times of the year (Pakiding, 2012). It has been reported for mixed pasture (grass/legume) that *L. perenne* produced 5613 tillers m^{-2} in Autumn compared with 4816 tillers m^{-2} in Summer (Yu et al., 2008), and a natural fertilised pastures registered 9372 tillers m^{-2} in Autumn compared with 8654 tillers m^{-2} in Summer for *L.perenne* (Chapter 2).

The combined effects of temperature and soil moisture in Autumn have strong influence on pasture production and may reflect a change on pasture species composition. Autumn rain is necessary to seedlings and legumes establishment before Winter. Also, soil Olsen P level must be taken into account to support species such as *L. perenne* and decrease *A. capillaris* content of the pasture. However, the increase of aluminium saturation affect negatively pasture growth, being necessary lime application (Chapter 1).

In Winter, when mean daily soil temperature decreases the growth rate of the pasture there is a slow down (Hennessy et al., 2008). During this season, leaf tissue turns over, leaf appearance rate and leaf senescence also slows down (Hennessy et al., 2006). From the point of view of soil aluminium saturation around 7.4% is considered high, constituting a permanent constraint to plant growth during the whole year around (Matu´sˇ et al., 2006). Winter production is restricted by low soil temperatures, which decrease soil microbial activity and plant metabolic activity.

The higher pasture yield was reached in spring with a possitive effect given by N fertiliser (40 kg N ha^{-1} season^{-1}), soil temperature and rain. The relationship between N, soil temperature and rain showed an important robustness in pasture production modelling on temperate climate. The pastures in southern Chile have seasonal periods of growth when N deficiency limits pasture growth (Chapter 1).

Pasture production responds to the N application throughout the year, with greatest responses after a Summer drought (Autumn) and next to cold/wet Winter. These responses are related with environmental and nutrient cycling. For example, in Autumn N deficiency is the result of a drought period. The first Autumn rain promote microbial breakdown of the organic matter that built up in Summer. The microbial activation is the responsible of N deficiency. N applied after the first Autumn rain helps to recover N deficiency created by soil microbial activity. After Winter N deficiency results from N leached from the wet soil. The strategic N application on Spring may stimulate the fast establishment of new species (Chapter 2).

In Summer to achieve a pasture production prediction of 2712 kg DM ha^{-1} is necessary a soil pH equal or more than 5.6 and soil mineral N equal or more than 50.8 mg kg^{-1} when there is drought conditions and high temperatures. The fertiliser applied in Spring would predict Summer production in terms of dry matter.

On the other hand, soil resistant to penetration is a factor associated to pasture productivity, it is an indicator of soil physical function as a degree of quality (Dörner et al., 2013). Soil penetration resistance depends on the soil water content, wich allows a good root development when there is less than 2000 kPa at field capacity (Horn and Fleige, 2009). The models selected the soil penetration resistance as a predictor variable of low pasture productivity on Spring, Autumn and Winter (predictive values of 1648, 2211 and 2385 kpa, respectively).

The soil penetration resistance registered on those times of year were 2122 kPa in Spring, 1679 kpa in Autumn and 1169 kpa in Winter, with a soil water of 20%, 36.1% and 36.5% v/v, respectively (Chapter 3). Studies showed that soil resistance decreases as a function of soil water content increase in a time scale (Lapen et al., 2004; Dec et al., 2011) and has been positively correlated with pasture yield (Dörner et al., 2013). However, sometimes, changes in the Pntr are not related with soil water content in grazing pastures due to animal hooves compact, deform, kneaded and homogenise the soil (Peth & Horn 2006; Dec et al., 2011; Krümmelbein et al., 2009; Dörner et al., 2013). Dec et al. (2011) concluded that grazing animals generates irregular soil compaction causing high spatial variability of penetration resistance not related to changes in water content.

The response to fertiliser and lime application on pasture production are positive in terms of fast growing species (Butkuviene & Butkute, 2008; Tharmaraj et al., 2008). There is a potential to increase annual and seasonal pasture productions providing a starting point to understand how could be the pasture production through decision tree modelling.

The Decision Tree model performed better than the Regression models with respect to model fit and provided insight if the input variables chosen are properly significant. Similarly, an application of the decision tree to predict species abundance in New Zealand pastures (Wan et al., 2009) and annual pasture productivity (Zhang et al., 2006). Decision tree models have been multipurpose, i.e. have been proved with remote sensed data to discriminate different tillage practices (Yang et al., 2003), climatic change (Prasad et al., 2006), precision agriculture (Waheed et al., 2006) or in natural resources management (Mbogga et al., 2010).

The evaluation of model prediction for annual and seasonal productivity had a high correlation between the predicted value and the observed value. The high R^2 found is considered an assessment of model performance (Pineiro et al., 2008; Smith and Rose, 1995). In other words, the decision tree models for annual and seasonal production were effective to predict pasture productivity.

The interacting effects of environment and pasture management on annual and seasonal production are of interest to producers and researchers. The reasonable agreement between pasture management variables, soil properties, climatic variables and decision tree models contributes to sustainable pasture development.

5. CONCLUSIONS

The decision tree models developed in this study revealed the importance of the environmental variables influencing annual and seasonal productivity in humid temperate pasture in temperate humid climates. Soil mineral nitrogen, pH and aluminum saturation were the variables with major influence on annual and seasonal growth, except by rain, which was the most important in Autumn. The decision tree model had better performance than models to predict pasture productivity with respect to model fit because had the highest correlation between the predicted and observed value.

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DISCUSIÓN GENERAL Y CONCLUSIONES

Existe una estrecha relación entre la condición de la pradera y la producción de materia seca (Nie et al., 2004; Tharmaraj et al., 2008; Capítulo 1). Las altas producciones han sido una característica de las praderas sembradas (Mixta y Diversa), sin embargo, la pradera naturalizada al alcanzar una buena condición, luego de corregir las limitantes nutricionales del suelo, puede alcanzar una producción similar o superior a la de praderas sembradas (Keim et al., 2014; Capítulo 1). Por el contrario, cuando una pradera está bajo estrés nutricional se ha medido que puede entregar producciones de 4-6 ton MS ha^{-1} $año^{-1}$ (Balocchi y López, 2009; Capítulo 1).

Son expresión del mejoramiento de una pradera el aumento de la abundancia de *Lolium perenne* y *Bromus valdivianus*. Ambas especies son frecuentes, tanto en praderas permanentes que fueron sembradas, como en praderas naturalizadas en buena condición (Capítulo 1; Tabla 2; López et al., 2013; Keim et al., 2014). Al contrario de *L. perenne* y *B. valdivianus*, que son especies que tenderían a dominar en condiciones de alta fertilidad (López y Valentine, 2003), las especies de hoja ancha poseerían una mayor capacidad para colonizar y permanecer en praderas bajo una diversidad de estados del suelo. Por ejemplo, es el caso de *Taraxacum officinale* G. H. Weber ex Wigg. e *Hypochaeris radicata* L. las cuales poseerían estrategias de crecimiento que les permitirían expresarse como competidores, oportunistas y tolerantes al estrés (Grime et al., 1989). Esto respalda la presencia de las especies de hoja ancha en las praderas evaluadas en este estudio. Por lo tanto, los atributos de una pradera, como son la composición botánica, la frecuencia, densidad y cobertura de las especies, obedecen a la interacción entre la capacidad de sobrevivir y desarrollarse de las especies pratenses y las condiciones del medio ambiente. Así, el medio ambiente y la pradera no pueden ser abordados en forma separada, están fuertemente relacionados, pero en el caso de los ecosistemas agrícolas, también condicionados por las estrategias de manejo aplicadas (Mysterud et al., 2011; Capítulo 4; Capítulo 5).

El pastoreo, a través de los criterios de pastoreo que se apliquen, pueden estimular el incremento de materia seca producida por una pradera y a la vez la sucesión de especies, favoreciendo el aumento de las especies de alto valor forrajero (Keim et al., 2014; Moir and

Moot, 2014). El ajuste de las cargas animales instantáneas obedece a la disponibilidad de materia seca de la pradera (Aydin y Uzun, 2005). Criterios de entrada de 8 a 10 cm (2.100 a 2.300 kg MS ha^{-1}) y de salida de 4 a 5 cm (1.000 a 1.200 kg MS ha^{-1}) (Matthews et al., 2004) estimularon dichos cambios (Capítulo 1, Tabla 4; Mackay et al., 2011; Fraser et al., 2011).

La intensidad de pastoreo es un factor que puede estimular a nuevos crecimientos en la pradera, como por ejemplo la generación de nuevos macollos para el caso de las gramíneas (Capítulo 2; Tabla 4), pero también puede afectar en forma negativa a la comunidad vegetal cuando se ejerce un sobrepastoreo (Stevens, 2011). En adición a esto, la capacidad de un animal de seleccionar materiales vegetales ejerce un efecto significativo sobre la presencia y crecimiento de las especies (López et al, 2003), siendo el ovino muy selectivo en el consumo de su dieta (Shakhane et al., 2013). En el estudio presentado en el Capítulo 1, especies como *L. perenne* y *B valdivianus* se vieron estimuladas para crecer como producto de la selección ejercida por los ovinos en pastoreo (Capítulo 1; Tabla 5). Es relevante considerar que dicho pastoreo fue controlado según los criterios de entrada y salida ya mencionados. En bovinos también se han registrado aumentos en el macollamiento de especies gramíneas como producto del pastoreo, pero con valores inferiores en la densidad de macollos en comparación a los medidos en praderas pastoreadas por ovinos (Nie et al., 2004; Li et al., 2006). Los resultados del pastoreo ovino en el presente estudio corroboran la utilización del pastoreo como una herramienta para el mejoramiento de praderas naturalizadas al alcanzar altas tasas de macollamiento y por la sucesión de especies de alta condición, tal como ha sido registrado en praderas del Hill Country en Nueva Zelandia (Smith, 2012).

La competencia entre especies pratenses está definida como el intento de plantas vecinas de utilizar las mismas unidades de luz, agua, nutrientes minerales o espacio (Grime, 1974), siendo un competidor considerado como un especialista en capturar recursos (Grace, 1991). Por lo tanto el proceso de competencia es de base genética, pero mediado por el ambiente, y cuyo resultado tiene su mayor expresión en la sucesión de especies en una pradera que se desarrolla con altos niveles de fertilidad de suelo (Grime et al., 1989). Bajo estas condiciones el factor ambiental que limita el crecimiento de las especies es la luz y la capacidad fotosintética de las especies y las tasas de crecimiento que desarrollan son las que determinan que especie dominará la pradera, es decir, cuál es el mejor competidor (Tilman, 1996). Especies competidoras como *L. perenne* se ven estimuladas en la producción de macollos al incrementar en nivel de nutrientes del suelo, mientras que, frente a esta condición, especies como *A. capillaris* disminuyen su densidad de macollos al aumentar su longitud de láminas (Capítulo 3; Figura 4).

La tolerancia al estrés es la capacidad de las especies pratenses de crecer y sobrevivir en un ambiente que posee una o más limitantes ambientales a su crecimiento (Grime, 1974). De esta forma una especie tolerante al estrés sobrevive en condiciones de alto estrés ambiental pero bajo nivel de alteración ecosistémica (Grime et al., 1989). Ejemplos de alto estrés ambiental son el pH ácido, baja disponibilidad de nutrientes minerales, alta saturación de aluminio en el suelo, entre otros. Especies tolerantes al estrés son *H. radicata*, *Anthoxanthum odoratum* L., *Leontodon nudicaulis* (L.) Banks ex Lowe, las cuales permanecen sobre la pradera a una tasa de crecimiento lento y constante, en circunstancias que las especies competidoras como *L. perenne* no son capaces de sobrevivir. Por lo tanto, es requisito para tener praderas de alta producción y calidad que las especies competidoras que la forman tengan satisfechos sus requerimientos internos de nutrientes (Pinochet, 2003) y suficiente estímulo de luz sobre sus yemas para generar nuevos crecimientos (Kami et al., 2010).

Por lo tanto, cuando las restricciones nutritivas del suelo son cubiertas y la competencia por luz es acentuada, *L. perenne* demostró su capacidad de crecimiento como competidor por sobre *A. capillaris* a través de la elongación de láminas y de la producción de macollos (Capítulo 3). La elongación de láminas evidenció la plasticidad de *L. perenne* ante la variación en la disponibilidad de luz, en el sentido que cuando comienza a hacerse restrictiva, ella tiene la capacidad de rápidamente ocupar los espacios disponibles para así ganar los espacios sobre el suelo, lo que interfiere negativamente a las especies vecinas de láminas más cortas y crecimiento postrado, como fue para el caso de *A. capillaris* (Capítulo 3).

La interacción y dinámica de las variables involucradas en el crecimiento de las especies pratenses pueden ser evaluadas en conjunto a través de la realización de los

Análisis de Árboles de Decisión, los que generan modelos de fácil interpretación, previa discriminación entre las variables, para su posterior jerarquización según su grado de afección sobre el crecimiento pratense (Moir et al., 2000). La aplicación de estos análisis a las praderas del sur de Chile constituye un aporte novedoso para la comprensión del funcionamiento y desarrollo de las mismas (Capítulo 5). Así el clima y el ambiente edáfico han sido indicados por estos modelos como las variables más importantes en afectar el rendimiento y la presencia de grupos funcionales de especies (Zhang et al., 2005; Wang et al., 2009; Capítulo 4; Capítulo 5).

Una propiedad de este análisis es que puede generar modelos para cada una de las estaciones y así priorizar y jerarquizar las variables climáticas ambientales que están determinando la producción pratense. Por ejemplo, el contenido de nitrógeno mineral del suelo fue indicado como una variable importante que influye en la productividad anual, primaveral y otoñal de la pradera, seguida de las precipitaciones y contenido de agua y temperatura del suelo (Capítulo 5; Figura 1, 2 y 4). La fertilización nitrogenada ha sido tradicionalmente reconocida como un importante input para la producción de la pradera (Pontes et al., 2007), y junto con las precipitaciones que ocurren en las épocas de crecimiento de la pradera (otoño y primavera), permiten generar el 60% de la producción anual (Capítulo 1).

Junto con el aumento de la producción y calidad, otro objetivo de una estrategia de mejoramiento de pradera es que, sobre la pradera en pobre condición, debiera aumentar la abundancia de especies de alta respuesta a la fertilización (HFRG; high fertility responsive grasses) (Wan et al., 2009). El aumento del contenido de nitrógeno disponible para las plantas en el suelo incrementa significativamente la abundancia de HFRG, mientras que en niveles bajos de fertilidad, las especies que se comportan como LFTG (low fertility tolerant grasses) y de hoja ancha pueden dominar una pradera (Wan et al., 2009; Capítulo 4, Figura 1 y 2). El fósforo disponible en el suelo para las plantas tiene un efecto similar al descrito para el nitrógeno en terminos de estimulación positiva de la sucesión ecológica pratense y así contribuye a través de procesos de competencia al establecimiento de especies de buen valor forrajero por sobre especies de baja y mediana respuesta a la fertilización. Un nivel de Olsen P que satisfaga los requerimientos de especies de altas tasas de crecimiento, como *L.*

perenne, influye positivamente en el incremento y persistencia de especies leguminosas como *Trifolium repens* L. (Fraser et al., 2011).

El Análisis de Árboles de Decisión también permite determinar no soólo cuáles son las variables más relevantes en estimular cambios sucesionales positivos en una pradera, sino que identificar a las variables que más limitan el crecimiento pratense y generan o son producto de procesos de degradación. Éste fue el caso del pH, el que fue indicado como la variable que más limitó la abundancia de leguminosas cuando se constató su disminución (Capítulo 4; Figura 3). Al contrario, cuando el pH aumentó, es decir fue menos ácido, la presencia de leguminosas incrementó, estuvo positivamente correlacionada con un pH superior o igual que 5.6. Es de esta forma como los Análisis de Árboles de Decisión generan modelos para determinar las variables involucradas en el proceso de progresión vegetacional y predecir así comportamiento en el tiempo, sino que también para determinar las causas que generan procesos de degradación y a la vez predecir las consecuencias de la sucesión negativa de las especies sobre las variables incidentes.

Los resultados y análisis de datos de la presente tesis confirman la importancia que tiene el suplir los requerimientos nutricionales de las especies pratenses, lo que constituye la base de toda sustentabilidad ecosistémica. Los ecosistemas de pradera tienden naturalmente hacia un balance entre lo que el suelo le entrega a la comunidad y la constitución y expresión de dicha comunidad. Pueden haber diferentes formas de entregar dichos nutrientes al suelo, ya sea a través de fertilizantes inorgánicos, como fue el caso de esta tesis, o por medio de fertilizantes orgánicos, pero lo relevante al final es que los nutrientes sean entregados al suelo en una cantidad y forma tal, que éstos sean disponibles para alcanzar la constitución y expresión pratense, como producción y calidad, propuestos como objetivo inicial.

Unido a lo anterior, el pastoreo intensivo es una herramienta para el mejoramiento de las praderas y para la sustentabilidad del sistema. En la presente tesis, la respuesta en el incremento de la producción de la pradera fue registrada como positiva desde el primer año, producto de la adición de fertilizantes y del control del pastoreo. El mejoramiento fue un proceso contínuo que se constató durante todo el período evaluado. Es muy posible que los cambios progresivos en la pradera y suelo hayan continuado, pero que cada año la diferencia de cambio en la pradera respecto del año anterior haya ido disminuyendo, debido a su acercamiento a la producción potencial del sitio, siendo el límite la condición climática del momento (interacción temperatura – precipitaciones).

Finalmente, en base a la hipótesis general planteada para esta tesis se concluye que el mejoramiento de una pradera naturalizada degradada, a través de la fertilización y del pastoreo intensivo ovino controlado, aumenta la abundancia de especies competidoras por nutrientes y luz, y producto de dicha progresión es el aumento en los atributos ecosistémicos – productivos deseables. Las especies competidoras al ser sometidas a pastoreo ovino intensivo controlado, son seleccionadas y estimuladas a aumentar su macollamiento y presencia en la pradera. Por lo tanto, el pastoreo incide en el mejoramiento sustentable de una pradera.

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