Effects of fire history on species richness and carbon stocks in a Peruvian puna grassland, and development of allometric equations for biomass estimation of common puna species

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Master of Science Thesis by Maarten van der Eynden

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Preface

This study was done as part of a project led by the University of Oxfords Environmental Change Institute investigating "The dynamics and carbon implications of fires in the Andes". Field work was carried out near the Wayqecha research station near the city of Cusco, Peru. The thesis was written at the Department of Ecology and Natural Resource Management at the Norwegian University of Life Sciences (UMB). Thanks to the Department of Ecology and Natural Resource Management at UMB, Lånekassen and Andreas og K. Ludvig Endresens Legat for funding the project.

I would like to take this opportunity to sincerely thank my two wonderfully helpful and patient supervisors. Thank you both for exchanging e-mail addresses when you met at a conference some years ago and thereby setting the stage for what at least I have found a very rewarding cooperation between the three of us. Thank you Torbjørn for honest comments and an always encouraging attitude. Thank you Imma for excellent guidance in the field, and for always keeping my spirit high, even when our samples went up in flames and fire research seemed like a very, very bad idea to an inexperienced Msc student.

I would also like to thank all the wonderful people who helped out with the fieldwork. Carlos Menor, Flor Zamora, Nelson Cahuana, Guissela Romani, Efrain Choque, Sandra Almeyda, Jose Kala, Jose Antonio and Walter Huaraca Huasco were all essential for the completion of this project. Thanks also to Matt Swan, Tim Hoolahan and all other students, researchers and staff I met in Cusco and Wayqecha. You all made this an unforgettable experience, filled with interesting conversations, music, dance and many breathtaking attacks of laughter.

Finally, I would like to thank my wonderful girlfriend Oda, my family and my friends. Thank you for all the good times along the way, and for always supporting me, although I seriously doubt that you have any idea about what I was really doing, measuring all that grass in Peru.

Abstract

High-elevation ecosystems have recently received increasing attention from the carbon financing sector. This has sparked the need for reliable and non-destructive methods to estimate carbon stocks in these ecosystems. The puna grasslands of the high Andes represent such a system and the current study investigated species richness and carbon stocks (in above- and below ground biomass) at a puna site in Peru. The study also examined the effect of fire on species richness and carbon stocks by comparing burnt and unburnt areas. Species-specific allometric equations were developed for four grass species, and generalised grassland equations were developed, combining data from both the burnt and unburnt area. No significant difference in carbon stocks between the burnt and unburnt area was found. The areas combined contained on average 3.4 Mg C ha^{-1} ± 0.1 SE stored in above-ground biomass, and 3.1 Mg C ha^{-1} ± 0.2 SE in below-ground biomass. Species richness was similar, but species composition differed somewhat between the burnt and unburnt area; the exotic species *Juncus balticus* was found mainly in the burnt area, and two *Lycopodium* species were found mainly in the unburnt area. However, *Calamagrostis sp.* was the dominant grass species in both areas. Highly significant allometric models were developed for four grass species separately. A generalised model combining the four was also developed. Some of the species-specific equations were affected by fire history. These results suggest that carbon estimations using allometric equations in puna grasslands can be more accurate if the fire history of the study area is known. It also seems that puna grasslands can recover their carbon stocks within three years of burning. However, species composition is altered by fire and appears to need more time to revert to pre-fire structure.

Key words: allometric equations, carbon, fire ecology, functional ecology, grasslands, Manu, mountains, Peru, puna.

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Introduction

The Andean grasslands in Peru (*puna*) have been exposed to increasing anthropogenic pressures during the last decades, mainly from grazing and burning (Bustamente Becerra & Bitencourt 2007; Tapia Nunez & Flores Ochoa 1984 in Bustamente Becerra & Bitencourt 2007). This could potentially lead to increased levels of soil erosion (Oscanoa 1988 in Bustamente Becerra & Bitencourt 2007), and consequently a decline in vegetation cover, primary production (Fensham 1997), plant diversity (Bustamente Becerra 2006), seed production and the amount of seeds stored in soil (Bertiller 1996; Coffin & Lauenroth 1989). It is also believed that grazing and burning of the puna in forest-puna transition zones constrains the upper limit of the tree line (Braun et al. 2002; Sarmiento & Frolich 2002; Young & León 2007). A serious concern considering that estimations for temperature rises during the next century may require species to migrate upwards at rates significantly higher than during the last 50,000 years (Bush et al. 2004; Feeley & Silman 2010). A balance between upwards migration of forests and conservation of puna biodiversity needs to be found.

Another aspect of fire and grazing pressure is their implications for carbon stocks. Anthropogenic land use is now widely considered to either contribute to carbon emissions through degrading land practices, or to function as a carbon sink for atmospheric carbon through sequestration in below- and above-ground forest- and grassland components (Denman et al. 2007). This has stimulated research on many different ecosystems with regards to global carbon dynamics, and their potential role in the recently developed carbon markets (e.g. Glenday 2006; Malhi & Grace 2000). Reforestation, avoided deforestation and better grassland management are some of the ways carbon credits for the voluntary carbon market can be generated (Hamilton et al. 2009), and even though most of the work until now has focused on lowland forest ecosystems, increasing attention is now given to carbon dynamics at higher elevations with the increasing recognition that these ecosystems also could benefit from carbon financing (e.g. Fehse et al. 2002; Malhi et al. 2010).

The frequent burning of the puna grasslands is likely to represent considerable emissions of carbon to the atmosphere, but little is known about puna carbon dynamics, especially in relation to fire (e.g. fuel build up). In one of the few studies conducted in puna areas, Gibbon et al. (2010) found that more carbon was stored in the soil of puna sites unaffected by fire than those that were fire exposed. However, the relationship was not statistically significant (Gibbon et al. 2010). No difference in carbon stored in above-ground biomass in relation to fire exposure was found either (Gibbon et al. 2010). In short, information on carbon dynamics under different disturbance regimes in puna areas is very sparse. More research is therefore clearly needed on puna carbon dynamics in relation to land use practices, and methods for estimation of carbon stocks need to be developed in relation to carbon trade.

Allometric equations have been shown to be an effective and non-destructive tool for estimating above ground biomass/carbon stocks (Chave et al. 2005; Litton & Kauffman 2008; Nafus et al. 2009). These equations can be species specific (e.g. Litton & Kauffman 2008) or more generalized (e.g. Chave et al. 2005; Nafus et al. 2009). Most of the existing equations focus on trees, since forests have received most of the attention with regards to carbon dynamics. The recent increased recognition that other ecosystems, such as grasslands, also contribute significantly to the global carbon cycle due to human land use (Scurlock & Hall 1998; Schuman et al. 2002), has sparked some interest in the development of allometric equations for these areas (e.g. Guevara et al. 2002; Nafus et al. 2009). For grasslands, generalised, species- and ecosystem-specific equations for dominant grasses and herbs are needed if carbon stocks are to be estimated precisely in the future.

The aims of this project were (i) to explore and quantify differences in carbon stocks of above- and below-ground biomass in an area exposed to fire three years previously and an area protected from fire for several years, (ii) to explore how the two areas differed in species richness and species composition and (iii) to develop allometric equations for the dominant grass species in the area for non-destructive above-ground carbon stock estimation in the future.

Methods

Site Description

The Manu National Park stretches from the Peruvian Amazon lowlands to the eastern slopes of the Andean mountains (IUCN 2008). The 1.5 million ha park is situated between the catchment basins of the Urubamba and Madeira rivers to the south and west, and the catchment of the Manu river in the eastern lowlands (Fig. 1; IUCN 2008). With an altitude gradient stretching from about 350 masl in the lowlands, to grasslands at around 4,000 masl, the park contains an extremely high diversity of habitats and species (IUCN 2008). Manu National Park was added to the World Heritage List in 1987 (IUCN 2008).

The study was conducted near the Wayqecha research station in the Manu buffer zone in the south-western mountainous part of the reserve at approximately 3300 masl (approximate coordinates 13^{o}18´S, 71^{o}58´W). The high altitude areas near Wayqecha have a typical puna vegetation type dominated mainly by tussock-forming grasses. Some of the dominant species are *Jarava ichu* Ruiz & Pav., *Calamagrostis vicunarum* (Wedd.) Pilg. and *Festuca dolichophylla* J. Presl. (Gibbon et al. 2010). Average annual rainfall is 1900 to 2500 mm, with a wet season from October to April (Gibbon et al. 2010). Mean annual temperature is approximately 11^{o}C (at 3600 m; Gibbon et al. 2010). Puna soils are largely composed of an organic-rich A-layer, stony B/C-layers, and no Oh-layer (Gibbon et al. 2010; Zimmerman et al. 2009). Gibbon et al. (2010)

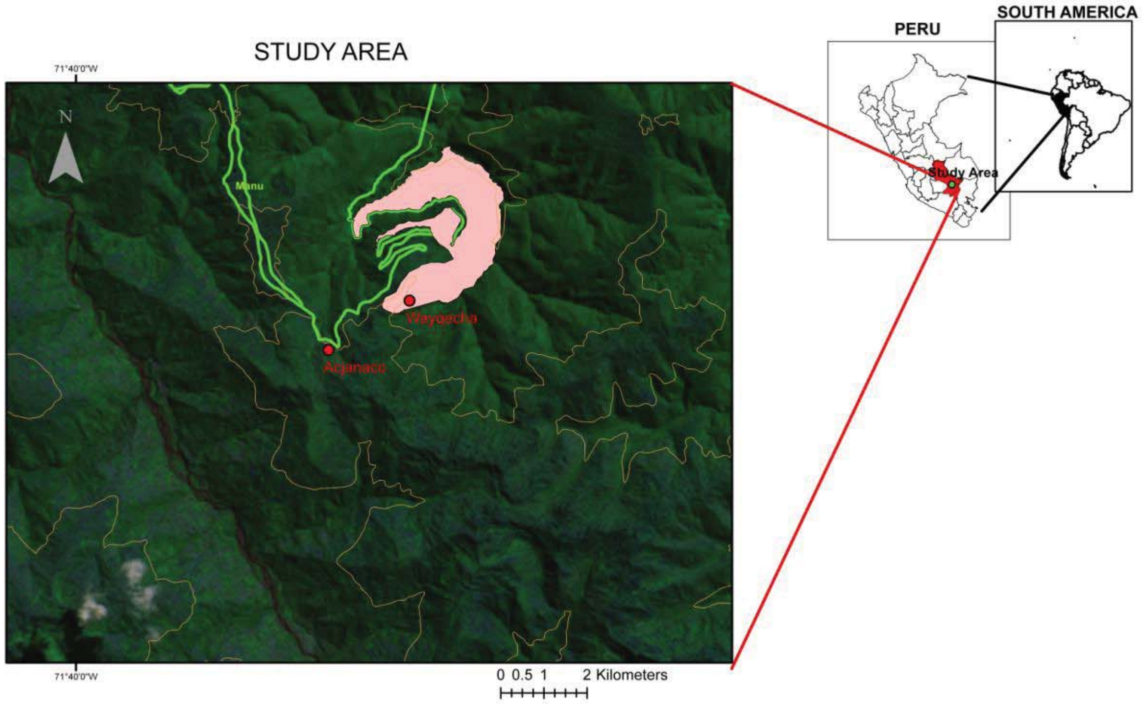


Figure 1: Map showing the location of the study area. The green border represents Manu National Park. The pink area represents the area of the Wayqecha Cloud Forest Research Centre, part of the southern buffer zone of Manu National Park.

reported a mean puna soil depth of 33 cm. The puna has been subject to high grazing and fire pressure over the years, and is classified as a "Zone of Recovery" by the Manu National Park, highlighting the need for spatial management for recovery (INRENA 2002 in Gibbon et al. 2010).

Above-ground sampling strategy

Eight transects of 30 m were set up (Fig. 2); four in an area burned in 2007 (Imma Oliveras *pers. comm.*) and four in an area unaffected by fire for at least ten years (Imma Oliveras *pers. comm.*). The sites are subject to similar grazing pressure. In each transect eight plots of 2 x 2 m were set up, each separated by two meters. All plants were identified to species level before the following measurements were taken for each tussock: The longest basal diameter and the longest perpendicular to the first (mm), the height as encountered in field (cm), the maximum height (stretched by hand; cm), the longest tussock crown diameter and the longest perpendicular to the first (cm). The crown- and basal diameters were averaged and used to estimate circular canopy areas and basal areas. The highest vegetative tiller was defined as plant height, excluding reproductive tillers that may surpass vegetative tillers. The biomass of these is negligible (Cavagnaro et al. 1983 in Guevara et al. 2002). Tussock volume was derived from plant heights and basal diameters using the "Basal Elliptical Cylinder" method as recommended by Johnson et al.

(1988; Fig. 3). All plants were hand clipped at ground level. Following Ramsay & Oxley (2001), dead material still attached to the tussocks was harvested, but ground litter was not. All plants were bagged and subsequently oven dried at 70°C to constant weight, and weighed to the nearest 0.1 g.

Below-ground sampling strategy

In each transect, four soil cores 12 cm in diameter and 30 cm in depth were extracted using opposable semicircular cutting blades. Small portions of soil were extracted at a time to avoid soil compression. The extracted soil was separated into an organic rich organic layer (OL) and a mineral layer (ML) and homogenised. Roots were extracted from each layer during four ten minute time intervals using one small plastic bag for the roots extracted per time interval. The soil was returned to the ground in its original layering after sampling. Following Girardin et al. (2010), the roots were transported to the lab where they were washed to remove inorganic material, separated into coarse roots (> 2 mm diameter) and fine roots (< 2 mm diameter), and dried to constant weight before they were weighed to the nearest 0.01 grams.

Carbon estimation

Dried vegetative biomass was assumed to contain 50 % carbon (following e.g. Gibbon et al. 2010; Glenday 2006).

Effect of fire on above-ground biomass and carbon stocks

Differences in total above-ground biomass between the burnt and unburnt area were compared using an independent samples t-test.

Effect of fire history on species richness and functional diversity

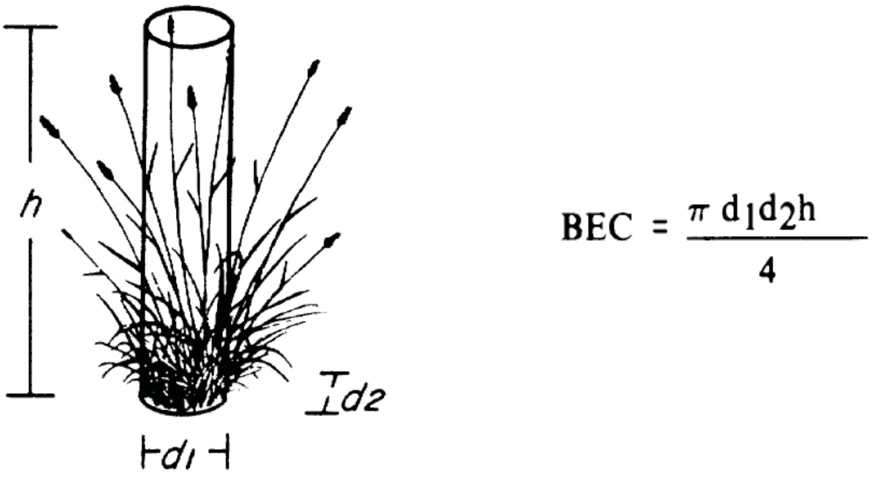
Plants in all plots were determined to species level and their biomass subsequently measured. Differences in plant biomass for the different species between the burnt and unburnt area were compared using independent samples t-tests.

Statistical analyses for below-ground biomass

The curve of cumulative root extraction over time was used to estimate root biomass that could potentially be extracted beyond 40 minutes for each soil sample, as shown by Metcalfe et al. (2007). This method corrects for the underestimation of below ground biomass often experienced in other

a *b* *c* *d* *e* *f*

Figure 2: Fieldwork images. *a* – setting up a transect. *b* **–** measuring a tussock. *c* – harvesting and marking a tussock. *d* – weighing samples. *e* – *Calamagrostis sp.* tussock. *f* – a 2x2 meter square after sampling.



$$BEC = \frac{\pi d_1 d_2 h}{4}$$

Figure 3: Canopy volume model calculated as a Basal Elliptical Cylinder using basal diameter and height measurements. Figure from Johnson et al. (1988).

methodologies (Metcalfe et al. 2007) and is much less time consuming without compromising measurement accuracy (Girardin et al. 2010; Metcalfe et al. 2007). The data obtained were used to estimate the amount of total biomass allocated below ground (in Mg/ha). The root biomass estimations were carried out using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, U.S.A.). Differences in total below-ground biomass between the burnt and the unburnt area were compared using an independent samples t-test.

Statistical analyses for above-ground biomass

Calamagrostis sp., *Festuca dolichophylla, Scirpus rigidus* and *Juncus balticus* have growth forms that make them suitable for developing allometric equations. These four species made up 84.9 % of the total biomass at the study site. The rest of the species have growth forms that make it difficult to explore allometric relationships with the methods used here, thus equations for these were not developed. Allometric equations were developed for the burnt and the unburnt area separately and for the two areas combined. A multispecies equation was also developed, using the data from both the burnt and unburnt area combined. The best models from the burnt and unburnt area were compared to determine if fire history affected the coefficients of the equations. If the 95 % confidence intervals of the coefficients overlapped, they were considered not significantly different. Because low numbers of *Juncus balticus* individuals were found, fire history specific equations were not developed for this species. All measured variables were $\log_{10}$ transformed in order to remove nonlinearity and heterogeneity of variance. Stepwise and simple regression was used to identify which variables influenced the model most and to identify possible co-linearity conflicts. Based on visual analysis of scatter-plots of estimators vs. biomass, the most extreme outliers (most likely annotation errors) were removed. However, the number of samples removed from the analysis was very low (<15).

Linear regression was performed to produce equations of the form

$Y = a + bX$

where Y = the log transformed dependent variable (plant biomass in grams), X = a log transformed independent variable (tussock volume, crown area, height or basal area) and a, and b are the regression coefficients derived from the linear regression analysis.

Some scatter-plots of independent variables vs. biomass suggested a more nonlinear tendency even after $\log_{10}$ transformation. Therefore, nonlinear regression was also performed to produce equations of the form

$Y = aX^b$

where Y = the log transformed dependent variable (plant biomass in grams), X = a log transformed independent variable (tussock volume, crown area, height or basal area) and a, and b are the regression coefficients derived from the nonlinear regression analysis.

Approximately 80 % of the data (called the estimation data set) were used to obtain the allometric relationships and 20 % (called the prediction data set) were used for validating the equations. Model accuracy was determined using the coefficient of determination (R^2), and the standard error of the estimate (SEE) with a higher R^2 and a lower SEE being a better fit than the opposite. Following e.g. Niklas (2006) analysis of residuals was also used. This was done through visual analysis of plots of predicted values against biomass residuals.

The addition of more than one independent variable to improve the equations was also explored, giving linear equations of the form

$Y = a + bX_1 + cX_2$

and

$Y = a + bX_1 + cX_2 + dX_3$,

where Y = the log transformed dependent variable (plant biomass in grams), X_n = a log transformed independent variable (tussock volume, crown area, height or basal area) and a, b, c and d are the regression coefficients derived from the linear regression analysis.

More variables were also added to the nonlinear models, producing equations of the form

$Y = aX_1^b + cX_2^d$

and

$Y = aX_1^b + cX_2^d + eX_3^f$

where Y = the log transformed dependent variable (plant biomass in grams), X_n = a log transformed independent variable (tussock volume, crown area, height or basal area) and a, b, c, d, e and f are the regression coefficients derived from the nonlinear regression analysis.

Some authors note that nonlinear regression techniques with untransformed data have often been used in studies of grass allometry (e.g. Johnson et al. 1988). The $\log_{10}$ transformed approach is used here because the raw data were not normally distributed, and because the analysis of residuals after exploring both techniques suggested a better fit for the $\log_{10}$ transformation method.

All analyses were carried out using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.) unless otherwise specified.

Results

Effect of fire on above-ground biomass and carbon stocks

The unburnt area contained more above-ground biomass than the burnt area, but the difference was not significant (P=0.27). The above-ground vegetation in both areas combined was estimated to contain on average 6.7 Mg ha^{-1} ± 0.2 SE dry biomass, which translates to 3.4 Mg C ha^{-1} ± 0.1 SE.

Effect of fire on functional diversity

The number of species in the burnt and unburnt plots was very similar (34 and 32, respectively). The species with the highest biomass was *Calamagrostis sp.* in both the burnt and unburnt area (Fig. 4). This species alone made up 71.5 % and 66.3 % of the total biomass in the burnt and unburnt area, respectively. However, there was no significant difference in *Calamagrostis sp.* biomass between the areas (P=0.994). Further*, Festuca dolichophylla* and *Scirpus rigidus* had a relatively high biomass in both areas, with more of both being found in the unburnt area (Fig. 4). However, these differences were not significant (P=0.162 and P=0.63 for *Festuca dolichophylla* and *Scirpus rigidus,* respectively).

The burnt area contained higher biomass of *Juncus balticus* (P=0.004)*, Baccharis pygmaea* (P=0.004) and *Blechnum sp.* (not significant P=0.282; Fig. 4). More biomass of *Senecio rhizomatosus* (P=0.003) and of two *Lycopodium* species was found in the unburnt than in the burnt area (one significant (P=0.035) and the other P=0.077; Fig. 4).

Effect of fire on below-ground biomass and carbon stocks

The unburnt area contained more below-ground biomass than the burnt area, but the difference was not significant (P=0.867). Few roots had diameters > 2 mm, and no roots were wider than ~ 4 mm. All the roots found were therefore treated as fine root biomass. The puna below ground vegetation was estimated to contain on average 6.3 Mg ha^{-1} ± 0.4 SE dry biomass, which translates to 3.1 Mg C ha^{-1} ± 0.2 SE.

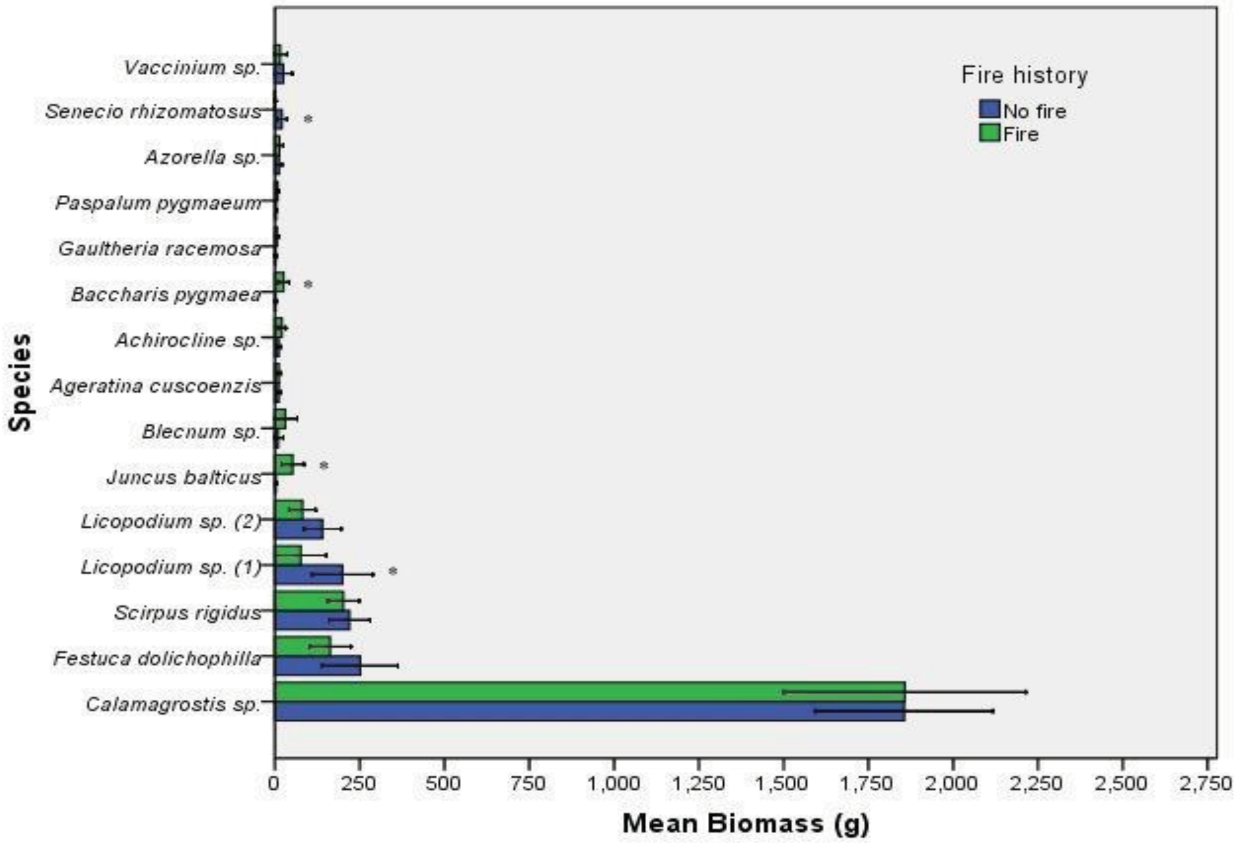


Figure 4: Mean biomass (grams per 2x2 meter plot) of a selection of species from a puna grassland, Peru. Blue bars indicate plants from an area protected from fire >10 years. Green bars indicate plants from an area burnt approximately three years before harvest. Error bars indicate 95 % confidence intervals of the mean. Asterisks indicate significant difference.

Allometric equations

Stepwise and simple regression techniques revealed that models based on basal area and height (either separately or as volume model) were good estimators of plant biomass. Basal area was the single most influential estimator, but adding height always improved the models. The addition of canopy area improved models in some cases, and in others not. The maximum height was a better estimator than height as encountered in field, and all models including height are therefore performed using the maximum height data.

Species-specific, fire history independent equations for both the estimation and the prediction datasets are presented in tables 1-4. Results for the burnt area are shown in tables 5-7 and for the unburnt area in tables 8-10. The comparison of coefficients based on the best fire history related models is presented in table 11. The results from the multispecies-multi area regressions are shown in table 12. All models had highly significant F-ratios ($p < 0.001$), and their residuals were determined to be approximately normally distributed.

Table 1: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Calamagrostis sp., and comparison of estimation and prediction data statistics. Equations combine data from a burnt and an unburnt puna site. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 1085, n prediction set = 249.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-1.274	0.792					0.759	0.301	0.279
Nonlinear	X1	0.091	2.196					0.794	0.277	0.268
Linear	X2	-0.751	0.696					0.813	0.266	0.244
Nonlinear	X2	0.195	1.706					0.835	0.248	0.23
Linear	X2 X3	-1.606	0.463	0.544				0.854	0.235	0.219
Nonlinear	X2 X3	0.257	1.529	-0.691	-1.9			0.838	0.246	0.23
Linear	X1 X4	-2.787	0.626	1.195				0.827	0.256	0.234
Nonlinear	X1 X4	0.242	1.587	-1.329	-2.703			0.818	0.26	0.244
Linear	X1 X3	-2.162	0.461	0.692				0.841	0.245	0.232
Nonlinear	X1 X3	0.202	1.695	-1.252	-1.578			0.805	0.27	0.258
Linear	X1 X4 X3	-2.744	0.464	0.494	0.661			0.855	0.234	0.298
Nonlinear	X1 X4 X3	0.266	1.526	-1.202	-2.775	-0.415	-1.732	0.819	0.26	0.244

Table 2: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Scirpus rigidus, and comparison of estimation and prediction data statistics. Equations combine data from a burnt and an unburnt puna site. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 508, n prediction set = 130.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.745	0.554					0.675	0.287	0.319
Nonlinear	X1	0.09	2.047					0.694	0.279	0.301
Linear	X2	-0.46	0.534					0.775	0.239	0.266
Nonlinear	X2	0.183	1.639					0.788	0.232	0.252
Linear	X2 X3	-1.49	0.363	0.522				0.838	0.203	0.223
Nonlinear	X2 X3	-93.919	-0.006	92.376	0.021			0.777	0.238	0.256
Linear	X1 X4	-2.42	0.473	1.192				0.808	0.221	0.225
Nonlinear	X1 X4	-341.264	-0.003	340.042	0.006			0.76	0.246	0.262
Linear	X1 X3	-1.993	0.327	0.685				0.807	0.222	0.24
Nonlinear	X1 X3	-2.474	-0.389	1.01	0.903			0.784	0.234	0.25
Linear	X1 X4 X3	-2.603	0.359	0.432	0.762			0.843	0.2	0.502
Nonlinear	X1 X4 X3	-416.124	-0.002	415.246	0.003	0.006	3.966	0.833	0.207	0.24

Table 3: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm*^{2} *(X1), tussock volume in cm*^{3} *(X2), canopy area in cm*^{2} *(X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Festuca dolichophylla, and comparison of estimation and prediction data statistics. Equations combine data from a burnt and an unburnt puna site. A higher R*^{2} *and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 183, n prediction set = 40.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.802	0.66					0.747	0.253	0.392
Nonlinear	X1	0.167	1.744					0.763	0.244	0.4
Linear	X2	-0.566	0.623					0.782	0.235	0.342
Nonlinear	X2	0.239	1.522					0.809	0.221	0.328
Linear	X2 X3	-1.231	0.508	0.333				0.801	0.225	0.327
Nonlinear	X2 X3	-308.246	-0.004	306.965	0.004			0.751	0.252	0.35
Linear	X1 X4	-3.021	0.557	1.382				0.804	0.224	0.327
Nonlinear	X1 X4	-213.656	-0.007	211.572	0.013			0.759	0.248	0.347
Linear	X1 X3	-1.669	0.492	0.463				0.787	0.233	0.356
Nonlinear	X1 X3	-167.114	-0.008	165.35	0.009			0.733	0.262	0.384
Linear	X1 X4 X3	-3.094	0.476	0.286	1.094			0.816	0.217	1.054
Nonlinear	X1 X4 X3	-265.389	-0.005	263.996	0.007	5.47E-005	7.194	0.815	0.219	0.363

Table 4: *Linear and nonlinear* regression *equations of different log transformed data (tussock basal area in mm*^{2} *(X1), tussock volume in cm*^{3} *(X2), canopy area in cm*^{2} *(X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Juncus balticus, and comparison of estimation and prediction data statistics. Equations combine data from a burnt and an unburnt puna site. A higher R*^{2} *and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 140, n prediction set = 37.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.641	0.488					0.665	0.289	0.311
Nonlinear	X1	0.067	2.215					0.68	0.282	0.308
Linear	X2	-0.361	0.475					0.743	0.254	0.266
Nonlinear	X2	0.166	1.686					0.75	0.25	0.242
Linear	X2 X3	-1.234	0.362	0.443				0.788	0.231	0.232
Nonlinear	X2 X3	0.285	1.277	-2.333	-3.266			0.771	0.24	0.24
Linear	X1 X4	-1.681	0.429	0.8				0.74	0.256	0.244
Nonlinear	X1 X4	0.397	1.078	-0.847	-1.42			0.718	0.268	0.268
Linear	X1 X3	-1.782	0.341	0.608				0.769	0.241	0.248
Nonlinear	X1 X3	1.023	0.544	-2.993	-1.146			0.739	0.256	0.273
Linear	X1 X4 X3	-1.982	0.349	0.466	0.359			0.778	0.237	0.248
Nonlinear	X1 X4 X3	59.299	0.013	-16.726	-0.032	-44.009	-0.03	0.749	0.254	0.248

Table 5: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Calamagrostis sp., and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site burned approximately three years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 575, n prediction set = 130.*

		Coefficient								Prediction
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	SEE
Linear	X1	-1.196	0.773					0.746	0.317	0.291
Nonlinear	X1	0.085	2.249					0.796	0.284	0.277
Linear	X2	-0.681	0.685					0.811	0.274	0.242
Nonlinear	X2	0.177	1.799					0.855	0.24	0.219
Linear	X2 X3	-1.634	0.434	0.597				0.861	0.235	0.207
Nonlinear	X2 X3	-0.809	-0.619	0.245	1.894			0.811	0.273	0.252
Linear	X1 X4	-2.647	0.58	1.233				0.83	0.259	0.236
Nonlinear	X1 X4	-272	-0.005	270.45	0.008			0.771	0.301	0.376
Linear	X1 X3	-2.204	0.426	0.756				0.85	0.243	0.214
Nonlinear	X1 X3	-1.468	-1.013	0.269	1.819			0.823	0.266	0.242
Linear	X1 X4 X3	-2.636	0.428	0.543	0.609			0.862	0.233	0.219
Nonlinear	X1 X4 X3	-372.855	-0.003	372.067	0.003	0.004	4.504	0.869	0.228	0.346

Table 6: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Scirpus rigidus, and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site burned approximately three years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 192, n prediction set = 56.*

		Coefficient								Prediction
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	SEE
Linear	X1	-0.815	0.603					0.704	0.293	0.3
Nonlinear	X1	0.096	2.055					0.728	0.282	0.298
Linear	X2	-0.547	0.595					0.8	0.241	0.272
Nonlinear	X2	0.18	1.727					0.821	0.228	0.275
Linear	X2 X3	-1.691	0.411	0.567				0.862	0.2	0.214
Nonlinear	X2 X3	-143.18	-0.005	141.376	0.015			0.816	0.232	0.234
Linear	X1 X4	-2.721	0.513	1.356				0.849	0.209	0.268
Nonlinear	X1 X4	-275.022	-0.005	273.559	0.008			0.814	0.232	0.291
Linear	X1 X3	-2.237	0.369	0.743				0.837	0.218	0.214
Nonlinear	X1 X3	-47.765	-0.019	45.448	0.048			0.817	0.232	0.223
Linear	X1 X4 X3	-2.871	0.412	0.419	0.892			0.874	0.192	0.684
Nonlinear	X1 X4 X3	-340.655	-0.003	339.437	0.004	0.021	2.971	0.859	0.204	0.216

Table 7: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Festuca dolichophylla, and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site burned approximately three years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 70, n prediction set = 15.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.869	0.701					0.758	0.251	0.154
Nonlinear	X1	0.181	1.718					0.77	0.248	0.164
Linear	X2	-0.57	0.645					0.785	0.236	0.167
Nonlinear	X2	0.251	1.515					0.798	0.232	0.173
Linear	X2 X3	-1.41	0.543	0.381				0.806	0.226	0.158
Nonlinear	X2 X3	-185.043	-0.008	183.335	0.008			0.773	0.246	0.216
Linear	X1 X4	-2.554	0.589	1.117				0.788	0.237	0.189
Nonlinear	X1 X4	-200.191	-0.009	198.241	0.012			0.759	0.254	0.207
Linear	X1 X3	-1.841	0.567	0.467				0.792	0.234	0.141
Nonlinear	X1 X3	-146.252	-0.011	144.017	0.011			0.766	0.25	0.167
Linear	X1 X4 X3	-2.803	0.52	0.354	0.79			0.805	0.229	0.641
Nonlinear	X1 X4 X3	-75.375	-0.02	73.745	0.021	0.01	3.303	0.786	0.242	0.176

Table 8: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Calamagrostis sp., and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site protected from fire >10 years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 514, n prediction set = 122.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-1.623	0.888					0.774	0.277	0.256
Nonlinear	X1	0.094	2.17					0.79	0.268	0.244
Linear	X2	-1.147	0.808					0.825	0.244	0.223
Nonlinear	X2	0.16	1.855					0.84	0.234	0.212
Linear	X2 X3	-1.871	0.554	0.522				0.861	0.218	0.221
Nonlinear	X2 X3	0.305	1.45	-1.818	-1.922			0.849	0.228	0.219
Linear	X1 X4	-3.655	0.703	1.506				0.836	0.237	0.225
Nonlinear	X1 X4	0.455	1.25	-2.908	-2.49			0.827	0.242	0.236
Linear	X1 X3	-2.361	0.533	0.666				0.841	0.232	0.238
Nonlinear	X1 X3	0.706	1.002	-3.073	-1.051			0.816	0.25	0.254
Linear	X1 X4 X3	-3.543	0.504	0.483	1.027			0.865	0.215	0.712
Nonlinear	X1 X4 X3	50.665	0.032	-20.38	-0.098	-33.448	-0.043	0.841	0.234	0.248

Table 9: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Scirpus rigidus, and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site protected from fire >10 years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 318, n prediction set = 85.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.793	0.561					0.683	0.281	0.311
Nonlinear	X1	0.077	2.156					0.715	0.268	0.296
Linear	X2	-0.529	0.548					0.774	0.238	0.244
Nonlinear	X2	0.148	1.803					0.803	0.223	0.232
Linear	X2 X3	-1.447	0.374	0.486				0.826	0.209	0.204
Nonlinear	X2 X3	-1.167	-0.569	0.332	1.519			0.773	0.238	0.232
Linear	X1 X4	-2.427	0.473	1.176				0.809	0.219	0.207
Nonlinear	X1 X4	-234.154	-0.005	232.819	0.008			0.767	0.242	0.248
Linear	X1 X3	-1.918	0.329	0.646				0.798	0.225	0.228
Nonlinear	X1 X3	-1.362	-0.95	0.204	1.851			0.775	0.238	0.236
Linear	X1 X4 X3	-2.576	0.362	0.388	0.796			0.837	0.202	0.559
Nonlinear	X1 X4 X3	-258.137	-0.003	257.3	0.004	0.001	5.385	0.842	0.2	0.221

Table 10: *Linear and nonlinear regression equations of different log transformed data (tussock basal area in mm^2 (X1), tussock volume in cm^3 (X2), canopy area in cm^2 (X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for Festuca dolichophylla, and comparison of estimation and prediction data statistics. Equations were developed with data from a puna site protected from fire >10 years before harvest. A higher R^2 and a lower SEE (standard error of the estimate) is considered a better fit. n estimation set = 110, n prediction set = 32.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-0.641	0.614					0.829	0.204	0.339
Nonlinear	X1	0.204	1.589					0.834	0.202	0.337
Linear	X2	-0.461	0.588					0.853	0.189	0.293
Nonlinear	X2	0.259	1.451					0.86	0.187	0.282
Linear	X2 X3	-0.652	0.548	0.104				0.855	0.189	0.279
Nonlinear	X2 X3	0.267	1.431	-0.036	-0.929			0.86	0.187	0.282
Linear	X1 X4	-2.834	0.538	1.309				0.861	0.185	0.244
Nonlinear	X1 X4	0.371	1.213	-30.087	-8.106			0.862	0.184	0.258
Linear	X1 X3	-0.967	0.538	0.188				0.836	0.2	0.309
Nonlinear	X1 X3	0.286	1.384	-0.406	-1.039			0.836	0.202	0.328
Linear	X1 X4 X3	-2.818	0.52	0.052	1.246			0.861	0.185	1.581
Nonlinear	X1 X4 X3	0.134	1.749	-5.57	-3.351	0.904	0.065	0.869	0.181	0.234

Table 11: *Comparison of regression coefficients from similar regression models based on data from one area burned approximately 3 years before harvest and one area protected from fire >10 years. Regression models were derived from different log transformed data (tussock basal area in mm*^{2} *(X1), tussock volume in cm*^{3} *(X2), canopy area in cm*^{2} *(X3), maximum plant height in cm (X4)), with a higher R*^{2} *and a lower SEE (standard error of the estimate) considered a better fit. Models are considered different if compared coefficients lie outside each other's 95 % confidence intervals.*

				Coefficient								
Species	Area	Regression	Estimator	a	95% interval	b	95% interval	c	95% interval	R^2	SEE	Prediction SEE
Calamagrostis sp.	Burnt	Nonlinear	X2	0.177	(0.159, 0.194)	1.799	(1.723, 1.875)			0.855	0.24	0.219
	Unburnt	Nonlinear	X2	0.16	(0.143, 0.178)	1.855	(1.774, 1.937)			0.84	0.234	0.212
	Burnt	Linear	X2 X3	-1.634	(-1.785, -1.483)	0.434	(0.393, 0.476)	0.597	(0.515, 0.678)	0.861	0.235	0.207
	Unburnt	Linear	X2 X3	-1.871	(-2.031, -1.711)	0.554	(0.502, 0.607)	0.522	(0.432, 0.611)	0.861	0.218	0.221
Scirpus rigidus	Burnt	Nonlinear	X2	0.18	(0.148, 0.212)	1.727	(1.578, 1.876)			0.821	0.228	0.275
	Unburnt	Nonlinear	X2	0.148	(0.126, 0.170)	1.803	(1.679, 1.926)			0.803	0.223	0.232
	Burnt	Linear	X2 X3	-1.691	(-1.954, -1.427)	0.411	(0.359, 0.464)	0.567	(0.447, 0.688)	0.862	0.2	0.214
	Unburnt	Linear	X2 X3	-1.447	(-1.649, -1.245)	0.374	(0.328, 0.419)	0.486	(0.388, 0.584)	0.826	0.209	0.204
Festuca dolichophylla	Burnt	Nonlinear	X2	0.251	(0.180, 0.322)	1.515	(1.292, 1.738)			0.798	0.232	0.173
	Unburnt	Nonlinear	X2	0.259	(0.213, 0.304)	1.451	(1.311, 1.591)			0.86	0.187	0.282
	Burnt	Linear	X2 X3	-1.41	(-2.085, -0.736)	0.543	(0.434, 0.652)	0.381	(0.098, 0.663)	0.806	0.226	0.158
	Unburnt	Linear	X2 X3	-0.652	(-0.992, -0.313)	0.548	(0.468, 0.628)	0.104	(-0.062, 0.269)	0.855	0.189	0.279
	Burnt	Linear	X1 X4	-2.554	(-3.694, -1.414)	0.589	(0.473, 0.705)	1.117	(0.390, 1.844)	0.788	0.237	0.189
	Unburnt	Linear	X1 X4	-2.834	(-3.725, -1.943)	0.538	(0.481, 0.595)	1.309	(0.786, 1.833)	0.861	0.185	0.244

Table 12: *Linear and nonlinear* regression *equations of different log transformed data (tussock basal area in mm*^{2} *(X1), tussock volume in cm*^{3} *(X2), canopy area in cm*^{2} *(X3), maximum plant height in cm (X4)) on log transformed plant dry biomass in g for four puna grass species combined (Calamagrostis sp., Scirpus rigidus, Festuca dolichophylla and Juncus balticus), and comparison of estimation and prediction data statistics. Equations were developed from plants found both in a puna site protected from fire >10 years before harvest and from a site burned three years before harvest. A higher R*^{2} *and a lower SEE (standard error of the estimate) is considered a better fit. n estimation data set = 1957, n prediction data set = 467.*

		Coefficient								
Regression	Estimator	a	b	c	d	e	f	R^2	SEE	Prediction SEE
Linear	X1	-1.216	0.756					0.737	0.333	0.334
Nonlinear	X1	0.072	2.339					0.779	0.306	0.317
Linear	X2	-0.781	0.691					0.813	0.281	0.281
Nonlinear	X2	0.147	1.912					0.851	0.25	0.258
Linear	X2 X3	-1.61	0.509	0.472				0.845	0.256	0.25
Nonlinear	X2 X3	0.167	1.824	-0.532	-2.658			0.852	0.25	0.258
Linear	X1 X4	-2.881	0.584	1.309				0.834	0.264	0.26
Nonlinear	X1 X4	0.281	1.483	-1.412	-2.407			0.817	0.279	0.281
Linear	X1 X3	-2.263	0.477	0.676				0.817	0.278	0.273
Nonlinear	X1 X3	0.16	1.826	-1.426	-1.88			0.789	0.298	0.308
Linear	X1 X4 X3	-2.984	0.479	0.374	0.934			0.851	0.251	0.731
Nonlinear	X1 X4 X3	0.286	1.47	-1.393	-2.412	-0.114	-2.221	0.817	0.279	0.279

Species-specific multi-area models

The linear model based on plant volume and canopy area gave the best fit for *Calamagrostis sp.*. However, when the predicted values were plotted against biomass residuals for this model, it seems that it underestimated biomass of small plants (Fig. 5). The nonlinear models based on volume and volume + canopy area also gave good fits, and give a better prediction for the full range of plant sizes (Fig. 6). These are therefore considered better than the linear model for *Calamagrostis sp.*.

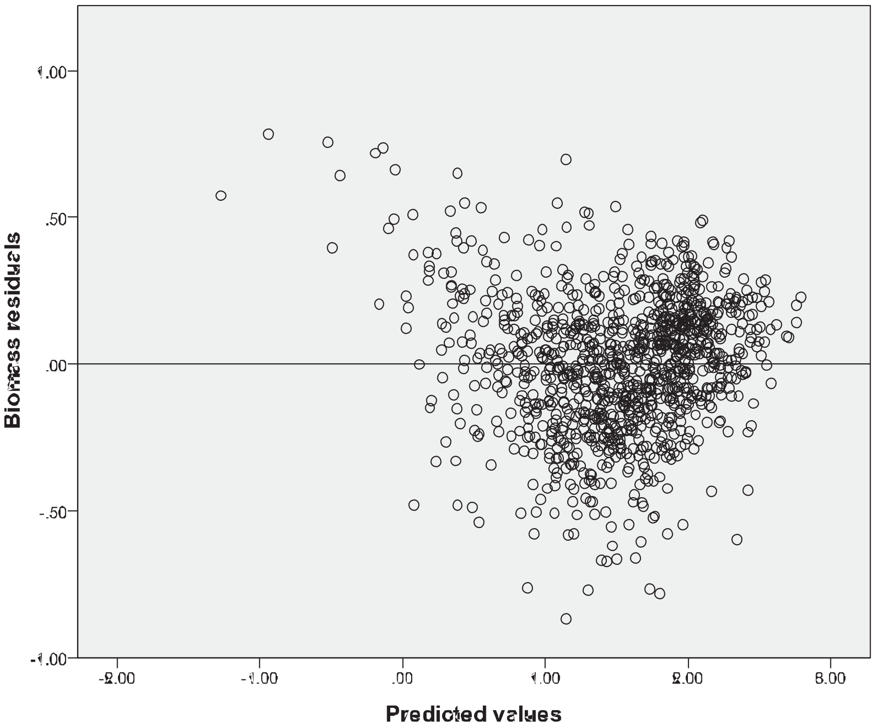


Figure 5: Plot of predicted values vs. biomass residuals for a linear allometric model based on volume and canopy area for *Calamagrostis sp.* in a puna grassland, Peru. The model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

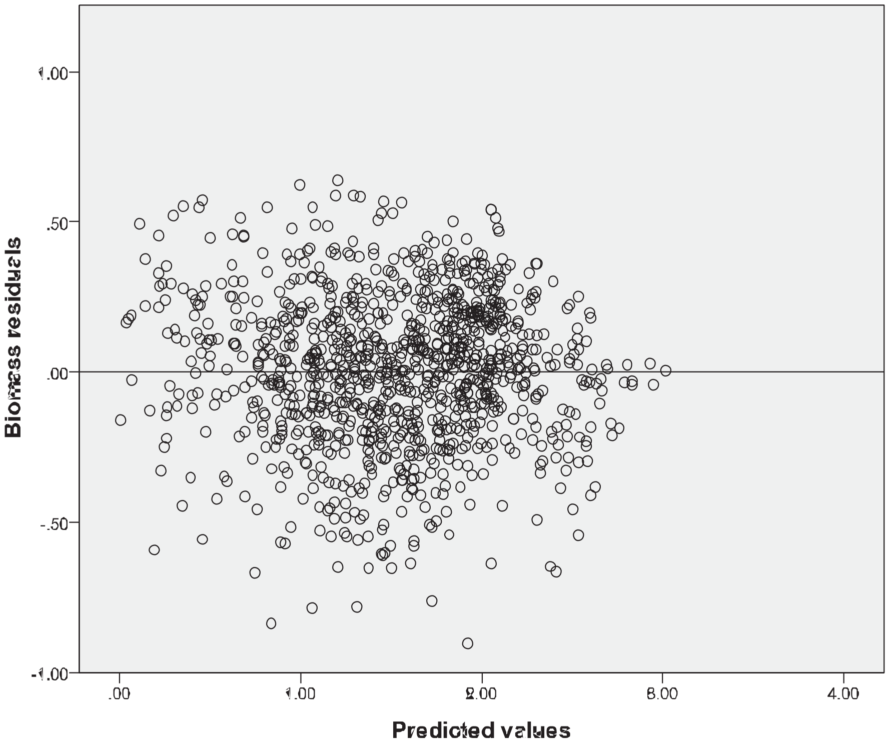


Figure 6: Plot of predicted values vs. biomass residuals for a nonlinear allometric model based on tussock volume for *Calamagrostis sp.* in a puna grassland, Peru. The model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

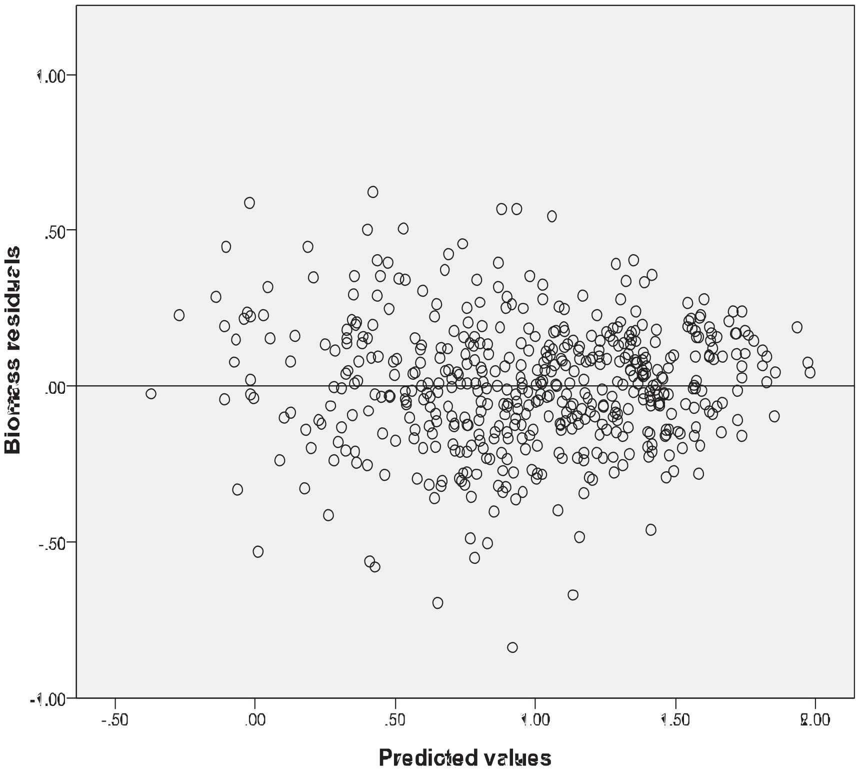


Figure 7: Plot of predicted values vs. biomass residuals for a linear allometric model based on volume and canopy area for *Scirpus rigidus* in a puna grassland, Peru. The model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

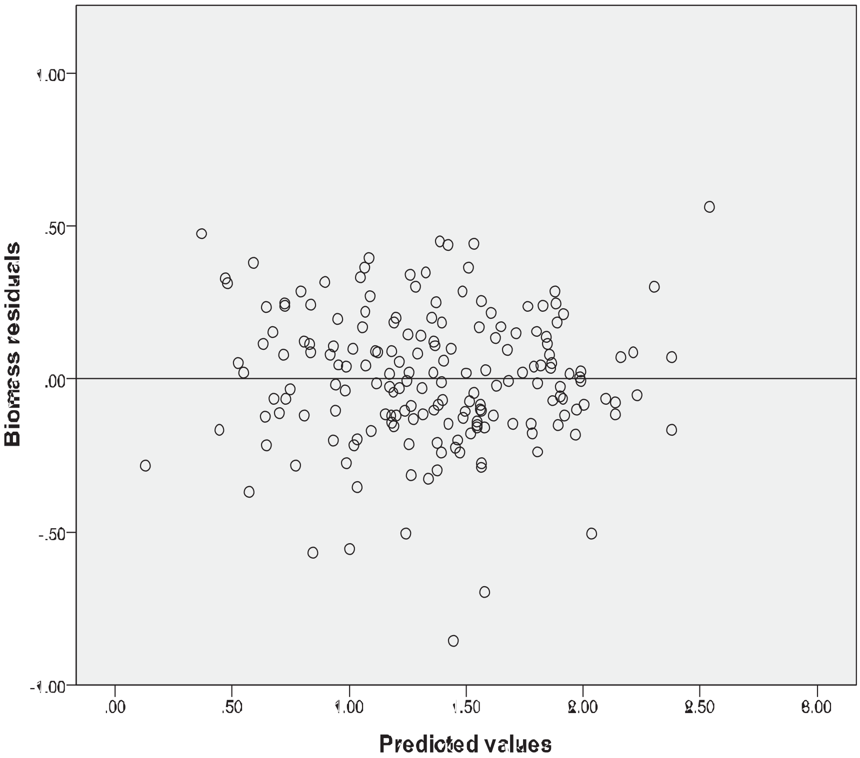


Figure 8: Plot of predicted values vs. biomass residuals for a nonlinear allometric model based on volume for *Festuca dolichophylla* in a puna grassland, Peru. The model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

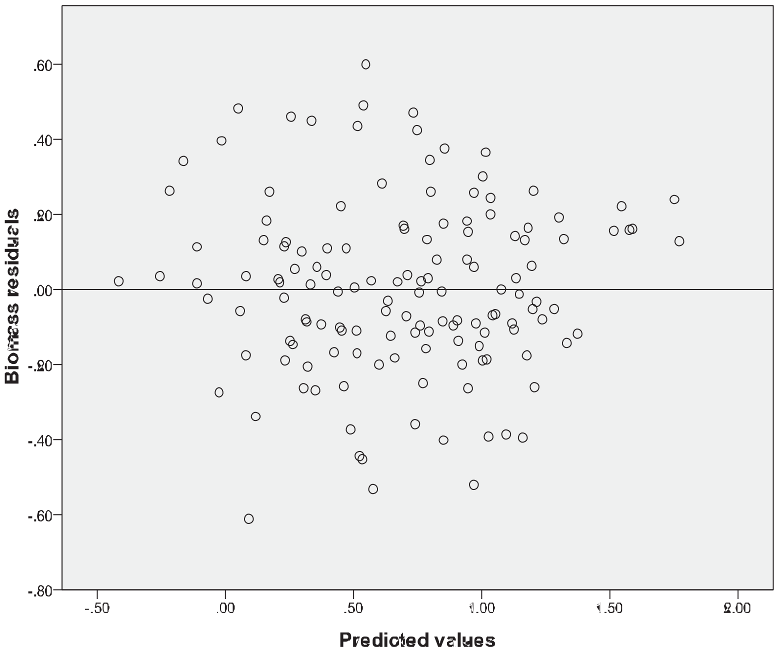


Figure 9: Plot of predicted values vs. biomass residuals for a linear allometric model based on volume and canopy area for *Juncus balticus* in a puna grassland, Peru. The model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

A linear model based on volume and canopy area gave the best fit for *Scirpus rigidus*. The plot of predicted values vs. biomass residuals shows that it gives a balanced estimation for the full range of plant sizes (Fig. 7).

The *Festuca dolichophylla* analysis showed good results for nonlinear models based on volume alone and for volume combined with canopy area. Plots of predicted values vs. residuals were good for both models. However, the prediction data fit was considerably better for the model based on volume alone, and this model is therefore considered better (Fig. 8).

The analysis of *Juncus balticus* showed that a linear model combining volume and canopy area was the best fit. The plot of predicted values vs. biomass residuals was also balanced (Fig. 9).

Multispecies model

The multispecies analysis based on data from *Calamagrostis sp., Scirpus rigidus, Festuca dolichophylla* and *Juncus balticus* data from the burnt and unburnt area combined, showed that nonlinear models based on volume and volume + canopy area gave the best fits. The residual analysis from the model based on volume also show a balanced estimation over the full range of plant sizes (Fig. 10). Adding canopy area only improves the R^2 value with 0.001 (Table 12).

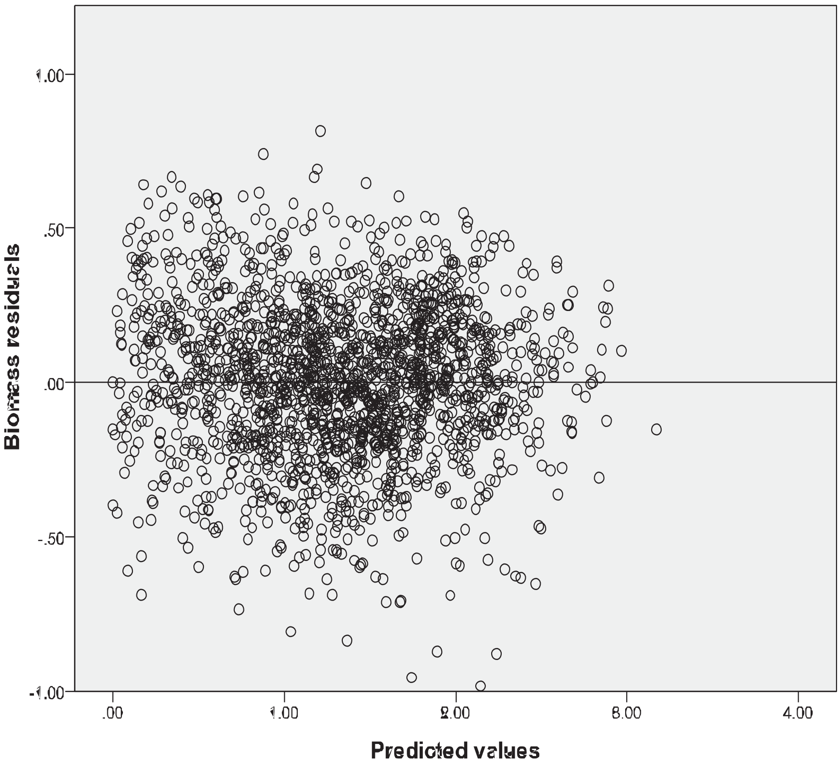


Figure 10: Plot of predicted values vs. biomass residuals for a nonlinear allometric model based on volume for *Calamagrostis sp., Scirpus rigidus, Festuca dolichophylla* and *Juncus balticus* combined in a puna grassland, Peru. Model is based on data from an area burned approximately 3 years before harvest and an area protected from fire >10 years combined.

Species-specific models based on fire history

The results from the analysis based on fire history corresponded with the multi-area models for *Calamagrostis sp.*. Linear models based on volume and canopy area gave better fits, but residual analysis show that nonlinear models based on volume and volume + canopy area produced a more balanced estimation across the full range of plant sizes. This was the case for both the burnt and unburnt area.

The *Scirpus rigidus* analysis gave similar results as the multi-area analysis, with a linear model based on volume and canopy area giving both the best fit and a balanced residuals plot in both the burnt and unburnt area.

For *Festuca dolichophylla* the results were somewhat different. In the burnt area, a linear model based on volume and canopy area gave the better fit, including balanced residuals. In the unburnt area a nonlinear model based on basal area, plant height and canopy area (as three independent variables) gave the best fit. However, it was only marginally better than a nonlinear model based on volume alone.

Many of the fire history based equation coefficients had overlapping 95% confidence intervals (Table 11). However, some of them did not, indicating equation differences between the burnt and unburnt area.

Discussion

Effect of fire on above-ground biomass and carbon stocks

Above-ground biomass did not differ significantly between the unburnt and the burnt area. Similar results were obtained by Gibbon et al. (2010). However, Gibbon et al. (2010) estimated above-ground carbon density at 6.5 Mg C ha^{-1} ± 0.3 SE for their grazed puna sites. This is significantly higher than the estimates presented here, where above-ground carbon density was 3.4 Mg C ha^{-1} ± 0.1 SE.

The difference between this study and Gibbon et al. (2010) could be explained by the fact that they also quantified the contribution of litter and moss to the carbon pool. They also operated in areas with some shrub cover. They fail to specify the contribution of litter and moss to the total carbon pool, but shrubs accounted for 0.5 Mg C ha^{-1} ± 0.2 SE.

The lack of effect of fire history may be related to short biomass recovery times. Biomass recovery in comparable ecosystems in Ecuador has been estimated to as little as 3-5 years (Ramsay 1992 in Ramsay & Oxley 1996), and physiognomic recovery in as little as 2 years (Ramsay & Oxley 1996). This also appears to be true for the burnt puna site in the current study because there was not significantly less biomass in the burnt than in the unburnt area investigated. However, Ramsay & Oxley (1996) also state that altitude influences recovery time, and that a biomass or physiognomic recovery does not necessarily mean that that community structure has completely recovered.

Ramsay & Oxley (2001) studied grazed Ecuadorian páramo grasslands and found carbon densities of 4.0 and 4.2 Mg C ha^{-1} at 3750 and 4000 meters, respectively. These values are comparable to those found in this study.

Hofstede et al. (1995) includes an overview of biomass studies carried out in a selection of high-altitude tropical and temperate grasslands. In general, páramo grasslands in Colombia appear to store more carbon in above-ground vegetation than other sites in tropical South-America (Hofstede et al. 1995). However, differences in sampling techniques make comparisons difficult.

Results from the present study correspond well to those obtained by Gibbon et al. (2010) in Peru and Ramsay & Oxley (2001) in Ecuador. Nevertheless, in order to obtain good estimations of carbon stocks in high altitude tropical ecosystems, more studies should be conducted - also in areas where a more detailed fire history can be obtained.

Effect of fire history on species richness and functional diversity

This is to date the first study to investigate the effects of fire on species richness in the puna ecosystem.

Species richness between the areas was relatively similar (34 and 32 in the burnt and unburnt area, respectively). *Calamagrostis sp.* was the dominant species in both the burnt and unburnt area, with 71.5 % and 66.3 % of the total biomass, respectively. This is in agreement with other studies in South-American tussock grasslands, where dominant grasses tend to re-colonise burnt areas quickly (Cianciaruso et al. 2010; Ghermandi et al. 2004; Laterra et al. 2003; Ramsay & Oxley 1996). Many species appear to be able to regenerate from roots or rhizomes, or may sufficiently shield developing buds from fire with less sensitive plant parts (Ramsay & Oxley 1996). Fire temperatures decrease rapidly down the soil profile (less than 65^{o}C 2 cm underground in Ecuadorian páramo; Ramsay & Oxley 1996), and can also be low enough at tussock bases for the survival of buds and rootstock (Ramsay & Oxley 1996). Interestingly, Trabaud (1987 in Ramsay & Oxley 1996) suggested that these shielding growth forms may be an adaptation to the cold Andean nights, more than a response to selective fire pressure.

Festuca dolichophylla and *Scirpus rigidus* also attained high biomass, with more of both being found in the unburnt area. This suggests that *Festuca dolichophylla* and *Scirpus rigidus* are inferior competitors and need more time to increase their biomass in an area. However, the differences were not statistically significant, which complicates any firm conclusions.

Juncus balticus was found mainly in the burnt area. This is an exotic species that has been known to colonise newly burned areas (Nelson Cahuana *pers. comm.*). The results therefore clearly support the hypothesis that the spread and/or persistence of exotic plant species can be facilitated by burning, as shown in several other South American ecosystems (Ghermandi et al. 2004; Gómez-Gonzales et al. 2011). However, it appears that *Juncus balticus* gradually loses its competitive advantage and is replaced by other species over time. This is reflected by the low biomass of *Juncus balticus* in the area not burned for several years. This strategy of colonising gaps created by disturbances is seen after fire in several ecosystems, and allows species to recharge the seed bank in anticipation of a new favourable disturbance (Ghermandi et al. 2004). However, some authors suggest that the small biomass of *Juncus balticus* in the unburnt area may be due to that a stable puna ecosystem might not provide an appropriate niche for its spread and growth (e.g. Shea & Chesson 2002).

Blechnum sp. had a higher biomass in the burnt area. This species is known as an indicator of past fire disturbance in puna ecosystems (Imma Oliveras *pers. comm.*) and this result was therefore to be expected. This difference was not statistically significant, but nearly all the *Blechnum sp.* biomass in the unburnt area was from one very large individual, while several individuals were found in the burnt area (*pers. obs.)*. The majority of biomass of two *Lycopodium* species was found in the unburnt area. These species grow as creeping "mats" on the ground between other plants, and plants with this growth form have been shown to be less fire resistant than tussock species in some ecosystems (Uys et al. 2004).

In addition, a higher biomass of *Senecio rhizomatosus* and *Baccharis pygmaea* was found in the unburnt and burnt area, respectively. Due to our poor understanding of many puna species, it is difficult to ascertain whether this is related to fire history. More studies on puna botany and ecology are clearly needed.

Effect of fire on below-ground biomass and carbon stocks

The average below-ground biomass of the whole study area was estimated to 6.3 Mg ha^{-1} ± 0.4 SE dry biomass, which translates to 3.1 Mg C ha^{-1} ± 0.2 SE.

No previous study has investigated below-ground biomass as a carbon pool in puna ecosystems. However, a simultaneous study from the Ajanaco ranger station located approximately 150 m higher than the current study site was found to contain 2.5 Mg C ha^{-1} (Oliveras *unpublished*). This corresponds well with results presented here, with no significant difference found when comparing data from Wayqecha and Ajanaco. Oliveras (*unpublished)* neither found significant differences between burnt and unburnt areas.

Zimmerman et al. (2010) investigated below-ground carbon stocks in puna, but did not separate below-ground biomass from soil organic carbon. They did not find significant differences in carbon stocks between their burnt and unburnt plots either, but comparison cannot be made directly as they did not separate biomass from their soil samples (Zimmerman et al. 2010).

Studies from other grassland and shrubland ecosystems present conflicting results on the effect of fire history. Cleary et al. (2010) and Coetsee et al. (2010) found no significant differences in fine root biomass based on fire history in a big sagebrush steppe in Wyoming, USA, and South African savanna, respectively, whereas Kitchen et al. (2009) found significant differences in a tallgrass prairie in Kansas, USA.

Ram et al. (1989) found from 1.6 to 2.4 Mg C ha^{-1} (recalculated from root biomass) in the Garwhal Himalaya, while Jackson et al. (1996) gives an average root carbon content of 3.9 Mg C ha^{-1} (recalculated from biomass) stored in the upper 30 cm of soil for tropical grasslands in their review of root distributions for terrestrial biomes. The corresponding number for temperate grasslands is 5.8 Mg C ha^{-1} (recalculated from root biomass). Nevertheless, the estimation of total root carbon ends up nearly equal due to the fact that more roots are stored below the upper 30 cm in tropical grassland soils than in temperate soils (Jackson et al. 1996).

The present study corresponds well with the above studies. However, comparisons are difficult due to the many different methods used and differences in ecosystems studied. In order to estimate carbon stocks more accurately, more below-ground biomass studies are needed in high-altitude tropical grassland ecosystems.

Allometric equations

The present study shows that basal measurements are the best predictor of above ground biomass for the species investigated. Adding height to the models improved them, while the addition of canopy measurements gave conflicting results. This agrees with Johnson et al. (1988), who found that a basal elliptical cylinder volume model based on basal- and height measurements gave a good estimation of plant biomass. They also found that adding canopy area to the equations improved them, but recommended the basal elliptical cylinder approach because canopy diameters can be difficult to measure correctly, especially in windy conditions. Both the results from the present study and those obtained by Johnson et al. (1988) show that basal area combined with height provide good estimations of plant biomass in the areas investigated. While Johnson et al. (1988) found that the addition of canopy measurements improved all their models, this was only the case for some species (*Calamagrostis sp., Scirpus rigidus* and *Juncus balticus*) examined in the current study.

Andariese & Covington (1986) and Nafus et al. (2009) found that basal measurements were the best estimator of above-ground biomass. They report small or no improvement when height is added to their models. Guevara et al. (2002) report that adding height in addition to basal area improved the model for *some* of the species investigated in an Argentinean grassland. The present study support basal measurements as the most influential estimator, but the conflicting results regarding addition of plant height and canopy measurements in different studies suggest that several parameters should be examined before conclusions are made.

Species-specific models based on fire history

Basal- and height measurements were good estimators of plant above-ground biomass in the species-specific models, and models could sometimes be improved by adding canopy measurements. However, the *Festuca dolichophylla* results show that estimators can vary in performance between areas based on fire history. Separate analyses should therefore be conducted in areas where differences in fire history are known in order to optimise results.

Comparison of model coefficients based on fire history

Comparing coefficients from the best models in the burnt and unburnt area revealed that some of them did not have overlapping 95 % confidence intervals. This confirms that fire history can affect regression coefficients.

Andariese & Covington (1986) also found that fire history could affect allometric models for grass species. They also found that differences in canopy cover could affect the models. Johnson et al. (1988) showed that regression coefficients could even vary from year to year in the same area.

In light of this, the results presented here confirm that allometric equations developed at a particular site should be used cautiously at different spatial and temporal scales since several factors can influence the equations. Whenever possible, equations should be developed annually, and several environmental factors should be known and considered (e.g. fire history, canopy cover, climatic measurements). A practical solution to these spatial and temporal challenges could be that the sampling area is increased to better account for variation between areas. Furthermore, the number of tussocks sampled in this study is significantly higher than what is normally sampled to develop allometric equations. This suggests that equations could be developed rapidly before carbon estimation is carried out, and that the method will be less destructive than it appears in the present study. Multi-year studies should also be conducted to see how the equations vary over time. Equations based on averages over several years could also potentially be useful in future carbon estimations.

Multispecies model

A nonlinear model based on canopy volume alone gave a very good fit with balanced residuals for the multispecies model. These results have several important implications for future estimates of carbon in puna grasslands using allometric equations. The fact that the model gives a good estimation without including canopy measurements could potentially save time and costs related to the extra work needed to take these measurements in field. In addition, uncertainties related to canopy measurements in windy conditions (as highlighted by Johnson et al. 1988) would be avoided. Furthermore, the good fit of the multispecies model eliminates the need for species identification skills and suggests that measurements may be conducted by untrained personnel. However, it must be stressed that this is only an advantage in general carbon stock measurements. In most cases, it would generally be of interest to know the species composition of the investigated area. As previously noted, this equation should also be applied to other areas with great caution, as the model may not be valid in areas with different species composition and environmental factors. However, the results show that multispecies grass models can be an important tool in future estimation of puna carbon stocks.

Conclusion

Puna carbon stocks in below- and above-ground biomass seem to be able to recover to pre-fire levels within three years. However, species composition was dissimilar, although the same species were dominant in both treatments. This suggests that fire alters species composition in puna grasslands, or that puna grasslands need more than three years to revert to pre-fire community structure. Future studies should focus on the effects of fire frequency and grazing pressure on both carbon stocks and species composition in puna areas in order to better understand carbon flow and the effects of anthropogenic pressures on this poorly studied ecosystem. Decreased pressure from fire and grazing at the tree line could also lead to increased income from future carbon trade (Gibbon et al. 2010). Studies aimed at managing the forest-puna transition zone in a way that allows for upward migration of tree species while still conserving puna biodiversity should therefore also be conducted.

Tussock basal area measurements combined with maximum plant height as a basal elliptical cylinder volume model proved to be a good estimator for above-ground grass biomass for dominant species, and the addition of canopy area to the models improved them further in some cases. These findings should facilitate future estimations of puna carbon stocks. However, the equations developed in this study should be implemented in other areas with caution, as several studies have shown that equations are both spatially and temporally specific (e.g. Andariese & Covington 1986; Johnson et al. 1988). Optimally, a new set of equations should be developed annually for more precise carbon stock estimation. Multi-year studies should also be conducted to explore how allometric relationships in grasslands change over time.

The lack of research concentrated on the puna grasslands of the high Andes clearly highlights the need for further studies on their ecology, and further studies are evidently needed to enhance our understanding of carbon flow in tropical mountainous ecosystems.

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