

SHORT COMMUNICATION

Variation in soil biological characteristics on an elevational gradient in the montane forest of north-west Argentina

Adriana B. Abril*¹ and Enrique H. Bucher†

* Microbiología Agrícola, Departamento de Recursos Naturales, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba.

C.C. 509, 5000, Córdoba, Argentina

† Centro de Zoología Aplicada, Universidad Nacional de Córdoba, Argentina

(Accepted 20 May 2008)

Key Words: altitudinal zonation, carbon cycling, decomposition, N-fixers, nutrient cycling

Montane tropical and subtropical rain forests are complex ecosystems, characterized by marked rainfall and temperature gradients with altitude, which in turn control the vegetation altitudinal zones (Hueck 1978). Montane forests are often referred to as cloud forests in recognition of the important influence of a dense and frequent cloud cover that conditions forest structure and functioning (Bautista-Cruz & del Castillo 2005, Holder 2004).

South American montane forests extend along the eastern slope of tropical Andes from Venezuela and Colombia to its southern limit in northern Argentina. In north-west Argentina they develop in the Sierras Subandinas mountain system and are locally known as 'yungas'. The forests range between 500 and 3000 m asl (Brown 1995, Grau *et al.* 2003, Hueck 1978). Three main vegetation altitudinal zones are clearly distinguishable: (1) The lower level, known as premontane forest (range: 400–1000 m asl) is a transition forest between the semi-arid woodland and the cloud forests. It is relatively diverse (20–30 tree species ha⁻¹), dominated by semideciduous, tall tree species including *Anadenanthera macrocarpa* (Benth.) Brenan, *Enterolobium contortisiliquum* (Vell.) Morong and *Phoebe porphyria* (Griseb.) Mez, with a dense understorey with abundant epiphytes and vines. (2) The intermediate level, known as montane cloud forest (range: 1000–1500 m asl), is a typical cloud forest dominated by evergreen species including *Myrcianthes pungens* (O. Berg) D. Legrand and *Blepharocalyx gigantea* Lillo, with great abundance of mosses and lichens both on the soil and trees. (3) The upper montane forest, known as

montane forest (range: 1700–2700 m asl), is a mosaic of relatively simple forest largely dominated by *Alnus acuminata* Kunth and *Podocarpus parlatorei* Pilg. with predominance of deciduous species and scarce epiphytes and a dense herbaceous cover.

Information on the montane forest soils of Argentina is limited in existing literature. Most of the available information refers to the premontane forest zone in areas deforested for agriculture. Of particular importance is the need for information on the biological processes that characterize these soils, taking into consideration that most of the available literature is restricted to soil structural and chemical characteristics (Aceñolaza & Gallardo-Lancho 1999).

An important question to be answered in the montane forests is the effect that elevational gradients and high level of soil water saturation have on soil dynamics, particularly in terms of organic matter decomposition and biological N₂ fixation, both highly dependent on environmental conditions such as availability of oxygen, nitrogen and carbon compounds (Abril & Bucher 1999, Abril *et al.* 2005, Kennedy & Bishop 2004).

We explore the working hypotheses that in the Argentine montane forests altitudinal changes in soil biological characteristics exert a strong influence generated by high cloud water deposition, rather than by the simple rainfall and temperature altitudinal gradient. Here we report a survey of soil biological characteristics (microbial biomass, micro-organism abundance, soil respiration and N₂ fixation) along an altitudinal transect in an undisturbed forest patch in north-west Argentina.

The study area is located in the Sierras Subandinas mountain system in Salta province (23° 04'–23° 8'S, 64° 19'–64° 51'W) of Argentina. Local climate is subtropical

¹ Corresponding author. Email: aabril@agro.uncor.edu

Table 1. Soil physical and chemical characteristics (mean \pm SD) at three altitudinal vegetation zones of Argentine montane forests. Letters indicate significant differences between sites (LSD test, $P < 0.05$).

| | Premontane forest | Montane cloud forest | Montane forest |
|--------------------------------------|-------------------|----------------------|-------------------|
| pH | 7.33 \pm 0.32 a | 7.00 \pm 0.17 a | 6.87 \pm 0.16 a |
| Organic matter (g kg ⁻¹) | 131 \pm 8.0 a | 74.5 \pm 5.4 b | 130 \pm 11.0 a |
| Total nitrogen (g kg ⁻¹) | 6.00 \pm 0.3 a | 3.53 \pm 0.81 c | 4.8 \pm 0.86 b |
| Nitrate (mg kg ⁻¹) | 91.6 \pm 5.35 b | 85.0 \pm 2.25 c | 110 \pm 4.03 a |
| Phosphate (mg kg ⁻¹) | 12.0 \pm 0.51 a | 7.28 \pm 1.10 c | 8.56 \pm 1.23 b |
| Conductivity (dS m ⁻¹) | 1.5 \pm 0.41 b | 3.9 \pm 0.51 b | 7.3 \pm 0.81 a |
| Sand (%) | 82.0 | 69.0 | 65.0 |
| Silt (%) | 15.0 | 25.0 | 28.0 |
| Clay (%) | 3.0 | 6.0 | 7.0 |
| Texture | Sandy-loam | Loamy-sand | Loamy-sand |

humid. Annual rainfall ranges between 1000 and 3000 mm, being concentrated between October and March. Both rainfall and temperature are strongly influenced by topography. Mean annual temperature at the lower elevation is about 23 °C. Predominant soils are well-drained, not very stony, derived from metamorphic rocks and Quaternary sediments.

We selected three sampling sites corresponding to each of the main altitudinal vegetation zones along the gas pipeline trail that connects Oran to Humahuaca. The locations for each zone include: (1) premontane forest (PF): El Oculito (500 m asl); (2) montane cloud forest (MCF): La Maroma (1000 m asl); and (3) montane forest (MF): San Andrés (1800 m asl). The selected sampling sites were not near either rivers or the pipeline trail.

During the first week of November 2001, 30 soil samples (0–20 cm deep) selected at random along 140-m-long transects were taken at each site. Soil samples were air dried for 24 h, sieved through a 2-mm mesh, and stored at 4 °C until processing within 48 h for biological parameters. It is known that cold storage may depress the absolute value of biological parameters, but does not affect relative patterns between samples (Verchot 1999).

The following soil parameters were analysed: soil moisture content by gravimetric method; organic matter (OM) content by the wet-digestion method of Walkley and Black (Nelson & Sommers 1982); total nitrogen by micro-Kjeldahl (Forster 1995); nitrate was extracted with KCl and measured using colorimetric methods (Keeney & Nelson 1982); available P (phosphate) by Bray method (Olsen & Sommer 1982), texture, conductivity, and pH, using the standard methods recommended by Soil Science Society of America (Klute 1986).

Microbial counts from each sample were made on a suspension of 1 g of soil in 100 ml saline solution (9%) shaken vigorously for 5 min. Abundance of microbial functional groups were estimated using the most probable number (MPN) and direct cell counts by agar plate methods. For the MPN method (three replicates) we used mineral salt basal medium containing: K₂HPO₄ (1.0 g

l⁻¹), MgSO₄·7H₂O (0.5 g l⁻¹) with selective substrate for each functional group as follows: ammonifiers, mineral media plus asparagine; nitrifiers, mineral media plus ammonium sulphate; and cellulose-degrading bacteria, mineral media plus cellulose strips (Lorch *et al.* 1995). For agar plate methods we used nitrogen-free basal medium (NFB) for N-fixing bacteria (Döbereiner 1995). The media pH was 7. Samples were incubated at 28 °C for 5–21 d, depending on the functional group.

Total heterotrophic activity was analysed through the CO₂ release method, in the laboratory under standardized soil conditions (Alef 1995a). Nitrogenase activity was determined using the acetylene reduction technique (Alef 1995b), incubating 1 g of soil for 24 h and microbial biomass C by the fumigation method (Joergensen 1995).

We estimated soil C balance by C mineralization ratio (CO₂/OM) (Abril *et al.* 2005) and fixed N₂ from ethylene production by the 3C₂H₄/1N₂ ratio (Werner 1995). Data from the different altitudinal vegetation zones were statistically analysed using ANOVA. We tested normality using the Kolmogorov–Smirnov test, and variance homogeneity using the Bartlett test. Means were compared using the least significant difference test (LSD) ($P \leq 0.05$ level) and correlation analysis was performed to test for linear relationships between variables.

In general, soils in the three study sites were sandy, non-saline, neutral in pH, and with a high organic matter and nitrogen content (Table 1). Organic matter, nitrate, total N and available P were significantly lower in MCF, whereas salinity was higher in MF and texture was sandier in PF (Table 1).

Microbial functional groups did not show a consistent relationship with elevation. Nitrifiers and cellulolytic organisms were significantly less abundant in the MCF ($P = 0.001$ and $P = 0.022$ respectively), whereas ammonifiers and N₂-fixing organisms did not show differences among sites (Figure 1). Soil respiration and microbial biomass were significantly lower in MCF ($P = 0.031$ and $P = 0.001$ respectively) (Figure 2). Both

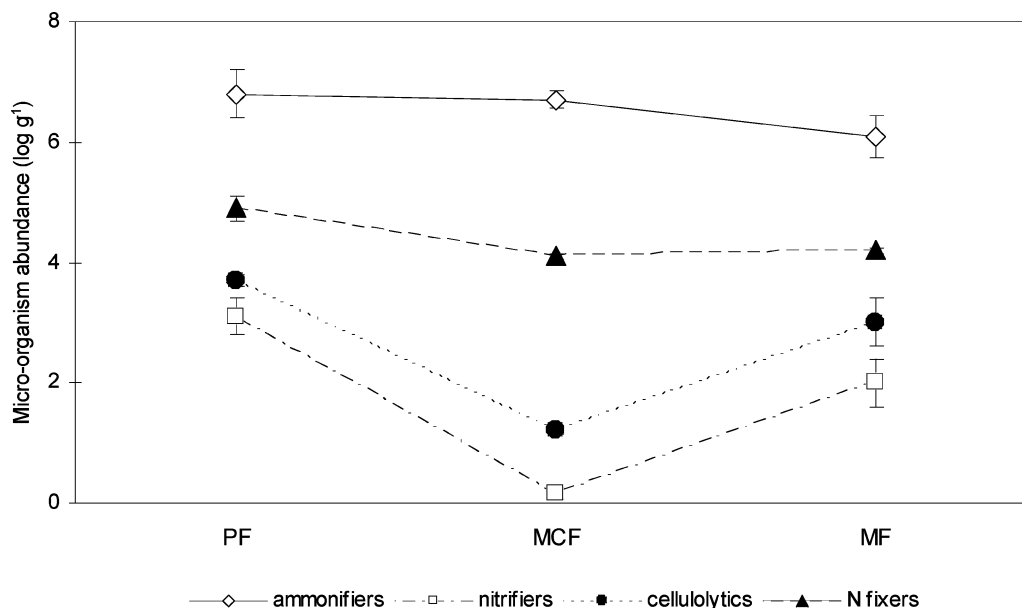


Figure 1. Micro-organism abundance (log bacterial number per g of soil) along an elevation gradient in three vegetation altitudinal zones of Argentine montane forests. PF: premontane forest; MCF: montane cloud forest and MF: montane forest. Error bars correspond to SE.

parameters correlated positively with organic matter content ($r^2 = 0.851$ and 0.964 , $P = 0.022$ and 0.001 respectively).

In contrast to the other studied parameters, N_2 fixation showed a significant increase in MCF soils ($P = 0.012$) (Figure 2). Nitrogenase activity correlated positively with N-fixing organism abundance ($r^2 = 0.850$, $P = 0.002$), and negatively with total nitrogen concentration ($r^2 = 0.838$, $P = 0.034$). C mineralization rate did not differ

among sites (Figure 2), suggesting a similar soil C balance in the three altitudinal vegetation zones.

The soil physical and chemical characteristics measured here are similar to those of other typical montane forests (Goller *et al.* 2006, Olander *et al.* 1998). The neutral soil pH values found at all altitudinal vegetation zones contrasts with other soils of South American tropical montane regions, generally characterized by very acid soils (Bautista-Cruz & del

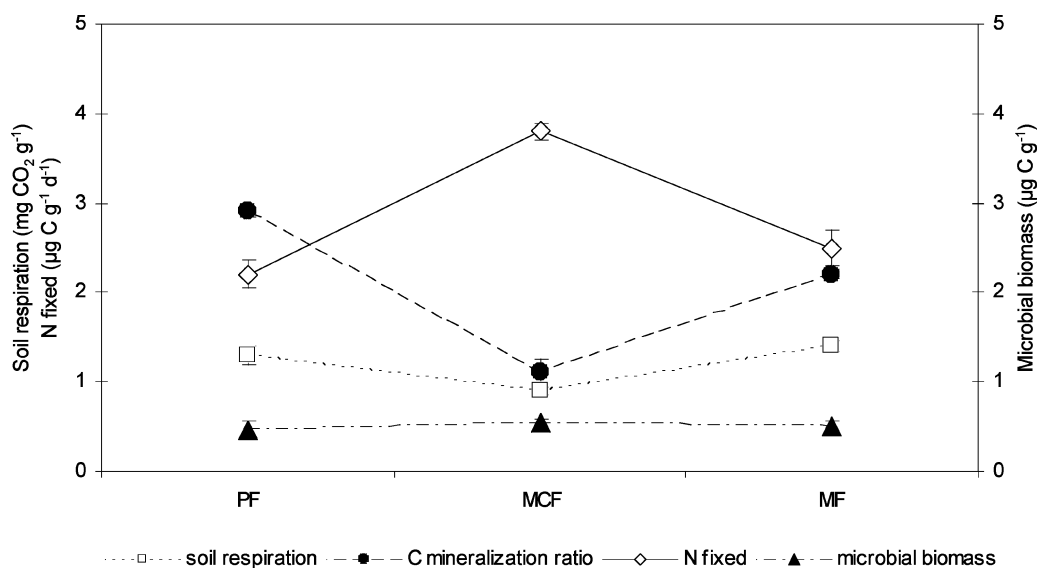


Figure 2. Soil respiration ($\text{mg CO}_2 \text{ g}^{-1} \text{ soil, 7 d}$), microbial biomass ($\mu\text{g C g}^{-1} \text{ soil}$), N_2 fixed ($\mu\text{g g}^{-1} \text{ d}^{-1}$) and C mineralization ratio along an elevation gradient in three vegetation zones of Argentine montane forests. PF: premontane forest; MCF: montane cloud forest; MF: montane forest. Error bars correspond to SE.

Castillo 2005, Olander *et al.* 1998, Sollins *et al.* 1994). This difference may be attributed to predominance of limestone sediments in the area, in contrast with the volcanic origin of other montane soils along the Andes range (Mingramm *et al.* 1979). With respect to P availability, information on montane forests is very limited. Our values are higher than those recorded in cloud forests of Venezuela (Bautista-Cruz & del Castillo 2005).

The studied soil parameters did not show a consistent altitudinal gradient. PF and MF vegetation zones are similar, with a marked discontinuity in the MCF zone, particularly regarding microbial parameters, soil organic matter content, total N and available nutrients (nitrate and phosphate). Coincidence in lower values of total heterotrophic activity, microbial biomass and abundance of strictly aerobic functional groups (cellulolytics and nitrifiers) would suggest that the lower soil organic matter values found at the MCF zone are the result of a restraint in the microbial litter decomposition process. This restriction could be due to added vertical (rainfall) and horizontal (cloud condensation) precipitation that results in waterlogged soils throughout the year (Hunzinger 1995, Nomura & Kikuzawa 2003). A water-saturated soil affects organic matter decomposition and nutrient release rates because of lower oxygen availability that decreases microbial activity (Bruijnzeel & Veneklaas 1998). A lower decomposition rate would explain a lower soil organic matter content as well as litter accumulation at the cloud forest vegetation zone (Hueck 1978, Kappelle *et al.* 1995).

A greater rate of biological N₂ fixation in the MCF could be explained by (1) a lower O₂ availability in waterlogged soils that favour nitrogenase enzyme activity, and (2) the lower total nitrogen availability in soil that induces activity of N₂-fixing organisms (Abril & Bucher 1999, Kennedy & Bishop 2004). Therefore, our observations are coincident with the statement that in tropical rain forests a significant proportion of soil nitrogen has an atmospheric origin (Parker 1994).

The results obtained in this paper, agree with our hypotheses that in tropical cloud forests the influence of cloud precipitation results in a unique discontinuity in the altitudinal gradient of soil biological characteristics not expected by rainfall and temperature changes alone.

ACKNOWLEDGEMENTS

We are grateful to Techint S.A. for helping us with the field work, and to S. Kopp and P. Torres for assistance with laboratory processing of samples.

LITERATURE CITED

ABRIL, A. & BUCHER, E. H. 1999. The effects of overgrazing on soil microbial community and fertility in the dry savannas of Argentina. *Applied Soil Ecology* 12:159–167.

- ABRIL, A., BARTTFELD, P. & BUCHER, E. H. 2005. The effects of fire and overgrazing disturbs on soil carbon balance in the dry Chaco forest. *Forest Ecology and Management* 206:399–405.
- ACEÑOLAZA, P. G. & GALLARDO-LANCHO, J. F. 1999. Leaf decomposition and nutrient release in the montane forest of north-western Argentina. *Journal of Tropical Forest Science* 11:619–630.
- ALEF, K. 1995a. Soil respiration. Pp. 214–219 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- ALEF, K. 1995b. Estimation of nitrogenase activity of free-living bacteria in soil. Pp. 234–245 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- BAUTISTA-CRUZ, A. & DEL CASTILLO, R. F. 2005. Soil changes during secondary succession in a tropical montane cloud forest area. *Soil Science Society of America Journal* 69:906–914.
- BROWN, A. D. 1995. Las selvas de montaña del Noroeste Argentino. Pp. 9–18 in Brown, A. D. & Grau, H. R. (eds.). *Investigación, conservación y desarrollo en selvas subtropicales de montaña*. Universidad Nacional de Tucumán, Tucumán, Argentina.
- BRUIJNZEEL, L. A. & VENEKLAAS, E. J. 1998. Climatic conditions and tropical montane forest productivity: the fog has not lifted yet. *Ecology* 79:3–9.
- DÖBEREINER, J. 1995. Isolation and identification of aerobic nitrogen fixing bacteria from soil and plants. Pp. 134–141 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- FORSTER, J. C. 1995. Soil nitrogen. Pp. 79–87 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- GOLLER, R., WILCKE, W., FLEISCHBEIN, K., VALAREZO, C. & ZECH, W. 2006. Dissolved nitrogen, phosphorus, and sulfur forms in the ecosystem fluxes of a montane forest in Ecuador. *Biogeochemistry* 77:57–89.
- GRAU, H. R., EASDALE, T. A. & PAOLINI, L. 2003. Subtropical dendroecology-dating disturbances and forest dynamics in northwestern Argentina montane ecosystems. *Forest Ecology and Management* 177:131–143.
- HOLDER, C. D. 2004. Rainfall interception and fog precipitation in a tropical montane cloud forest of Guatemala. *Forest Ecology and Management* 190:373–384.
- HUECK, K. 1978. *Los bosques de sudamérica. Ecología, composición e importancia económica*. GTZ, Eschborn. 476 pp.
- HUNZINGER, H. 1995. La precipitación horizontal: su importancia para el bosque y a nivel de cuencas en la Sierra San Javier, Tucumán, Argentina. Pp. 53–58 in Brown, A. D. & Grau, H. R. (eds.). *Investigación, conservación y desarrollo en selvas subtropicales de montaña*. Universidad Nacional de Tucumán, Tucumán, Argentina.
- JOERGENSEN, R. 1995. The fumigation extraction methods. Pp. 293–314 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- KAPPELLE, M., VANUFFELEN, J. G. & CLEEF, A. M. 1995. Altitudinal zonation of montane forests along two transects in Chirripo National Park, Costa Rica. *Vegetatio* 119:119–153.
- KEENEY, D. & NELSON, D. 1982. Nitrogen inorganic forms. Pp. 643–698 in Page, A. L., Milles, R. H. & Keeney, D. R. (eds.). *Methods of soil*

- analysis. American Society of Agronomy and Soil Science Society of America, Madison.
- KENNEDY, C. & BISHOP, P. 2004. Genetics of nitrogen fixation and related aspects of metabolism in species of *Azotobacter*: history and current status. Pp. 27–52 in Klipp, W., Masepohl, B., Gallon, J. R. & Newton, W. E. (eds.). *Genetics and regulation of nitrogen fixation in free-living bacteria*. Kluwer Academic Publishers, Dordrecht.
- KLUTE, A. (ed.). 1986. *Methods of soil analysis*. Vol. 1. *Physical and mineralogical methods*. American Society of Agronomy and Soil Science, Madison. 980 pp.
- LORCH, H. J., BENCKIESER, G. & OTTOW, J. C. 1995. Basic methods for counting microorganisms in soil and water. Pp. 146–161 in Alef, K. & Nannipieri, P. (eds.). *Methods in applied soil microbiology and biochemistry*. Academic Press, London.
- MINGRAMM, A., RUSSO, A., POZZO, A. & CAZAU, L. 1979. Sierras Subandinas. Pp. 95–137 in *Geología regional argentina*. Academia Nacional de Ciencias, Córdoba.
- NELSON, D. W. & SOMMERS, L. E. 1982. Total carbon, organic carbon and organic matter. Pp. 570–574 in Page, A. L., Milles, R. H. & Keeney, D. R. (eds.). *Methods of soil analysis*. American Society of Agronomy and Soil Science Society of America, Madison.
- NOMURA, N. & KIKUZAWA, K. 2003. Productive phenology of tropical montane forests: fertilization experiments along a moisture gradient. *Ecological Research* 18:573–586.
- OLANDER, L. P., SCATENA, F. N. & SILVER, W. 1998. Impacts of disturbance initiated by road construction in a subtropical cloud forest in the Luquillo Experimental forest, Puerto Rico. *Forest Ecology and Management* 109:33–49.
- OLSEN, S. R. & SOMMER, L. E. 1982. Phosphorus. Pp. 403–430 in Page, A. L., Milles, R. H. & Keeney, D. R. (eds.). *Methods of soil analysis*. American Society of Agronomy and Soil Science Society of America, Madison.
- PARKER, G. G. 1994. Soil fertility, nutrient acquisition and nutrient cycling. Pp. 54–64 in McDade, L. A., Bawa, K. S., Hespeneide, H. A. & Hartshorn, G. S. *La Selva: ecology and natural history of a neotropical rain forest*. University of Chicago Press, Chicago.
- SOLLINS, P., SANCHO, F., MATA, R. & SANFORD, R. L. 1994. Soil and soil process research. Pp. 34–53 in McDade, L. A., Bawa, K. S., Hespeneide, H. A. & Hartshorn, G. S. *La Selva: ecology and natural history of a neotropical rain forest*. University of Chicago Press, Chicago.
- VERCHOT, L. V. 1999. Cold storage of a tropical soil decreases nitrification potential. *Soil Science Society of America Journal* 63:1942–1944.
- WERNER, D. 1995. *Symbiosis of plants and microbes*. Chapman & Hall, London. 389 pp.