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SHORT COMMUNICATION

Accounting for fine root mass sample losses in the washing process: a case study from a tropical montane forest of Colombia

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Fine roots are very important in ecology because of their role in nutrient and water uptake, and as a source of organic matter to the soil. In carbon-cycle models, fine roots are a significant organic matter pool (Potter 1999) with high net primary productivity (Jackson *et al.* 1997) and turnover (Gill & Jackson 2000). In ecosystem production studies, fine roots are usually divided between biomass and necromass. The sum of both pools is defined as root mass (Klinge & Herrera 1978) or total root biomass (Böhm 1979). In tropical forests the study of fine-root biomass is restricted because of the difficulties in distinguishing live roots. Visual methodologies are not adequate in tropical forests where high diversity is expressed through many root morphologies. On the other hand, definitions of root death are ambiguous and differ between different studies (Comas *et al.* 2000). Fine-root mass is easier and more accurate to measure than fine-root biomass because subjective selection criteria are avoided. However, in the measurement of below-ground production, the estimation of fine-root biomass is essential (Jackson *et al.* 1997). The adaptation of objective selection methods (Comas *et al.* 2000, Joslin & Henderson 1984) to measure live and dead root fractions is urgently needed.

Among the most important sources of error in the estimation of root mass are losses of root material from soil core samples at washing and sorting. Bakker (1999) found that these losses were around 20% on a dry-weight basis. In this paper we assess fine-root ($\leq$ 5 mm) mass losses from soil core samples from a tropical forest in Colombia.

The study was carried out in two sampling plots located at the mid-watershed of the Porce River (6°45′37″N, 75°06′28″W), Colombian Andean mountains, in December 1999. Mean annual rainfall is 3050 mm, mean annual temperature is 21.8 °C and altitude approximately 1200 m asl. Soils have low fertility, high acidity ($4.6 < pH < 5.0$) and have been classified as Ustoxic Dystropept (Jaramillo 1989). Mean bulk density is 1.1 g cm^{-3} (Lara 2003). The sampling plots (20-m × 50-m) were located in a primary rain forest. Seven samples 0–30 cm depth were taken at random locations in each plot using a bi-partite root auger (Eijkelkamp, Giesbeek, the Netherlands), 8 cm diameter and 15 cm length. Two soil cores were extracted per sample location, one at 0–15 cm depth and then, in the same hole, the other at 15–30 cm depth. Each soil core was labelled and stored in plastic bags. Then, in the laboratory each core section was processed by washing and sieving the content sequentially through 4.75-, 2-, 1.4- and 0.85-mm sieves. All visible fine roots ($\leq$ 5 mm) were hand sorted using forceps and measured using a digital calliper. Although the limit between fine and coarse roots is ambiguous, we use 5 mm as in many other studies (e.g. in the global database compiled by Gill & Jackson (2000), 20 references used a limit of 5 mm, 16 references 2 mm, and 15 references 3 mm).

All remaining material after sieving was collected in a fabric-mesh sieve (0.3–0.5 mm opening diameter). This material is called residuals (RES), which includes very fine roots (< 0.5 mm), root fragments, sand and some silt. It is assumed that in the washing process all clays, humic and fulvic acids were removed from the material collected in RES. Separation of very fine roots from the material remaining on the fabric mesh was not possible. All fine roots collected were oven dried at 80 °C and weighed. RES were air dried, weighed, oven dried and weighed again. Three subsamples of RES for each soil-core section

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were burnt at 500 °C. Ash-free RES were weighed, and the difference was considered to be the amount of very fine roots remaining in RES after washing, i.e. a quantity that could not be accounted for. In order to account for any soil organic matter (OM) remaining in RES, five subsamples not used for the ashing were analysed by the Walkley–Black method. Mean OM was 0.16%, and this figure was used as a correction factor.

Results show a great difference (t-test, $P < 0.001$) between estimates with and without the mass of very fine roots in RES (Table 1). Approximately 7.2 t ha^{-1} could be underestimated (39.2%) through losses in the washing process. This quantity could be higher if a finer sieve were used. The coefficient of variation tends to be similar in both cases; this means that RES variability is proportional to the stock of fine roots in each site.

According to the simple random sampling (the sampling design used in this study), the distribution of the variable studied must follow a bell-shaped distribution (Tryfos 1996). In Figure 1, the frequency histograms for both variables, fine roots with and without RES, are shown. It is clear that the sampling without very fine roots does not have a bell-shaped distribution. The normality test applied to the data (Shapiro–Wilks test, $\alpha = 0.05$) (Conover 1980), indicates that only the sample with RES can be adequately modelled by a normal distribution with 95% accuracy. If the material collected in RES were not included, the sample mean and variance would not satisfactorily explain the properties of the population (Sheskin 2000, Tryfos 1996).

In order to validate this result, a second measurement was taken in the same plots at different sampling points 2 mo later. Fine root mass with very fine roots accounted for 17.0 ± 5.2 t ha^{-1} and the amount of fine root mass measured in RES was 53.9%. Frequency distributions showed the same patterns as in Figure 1.

According to these results, we can hypothesize that some previous studies could have underestimated fine root mass because their measurements did not include a procedure to diminish the losses of root material in the washing process. This error affects global estimates for belowground biomass in forest ecosystems (Cairns *et al*. 1997, Jackson *et al*. 1996, Sanford & Cuevas 1996). The procedure suggested in this paper was developed in soils without carbonate carbon. Soils with high carbonate carbon concentrations require a correction factor including the inorganic carbon content.

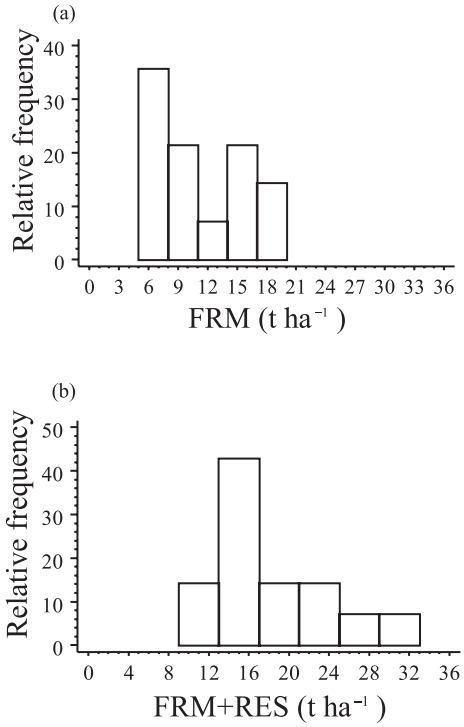

Figure 1. Frequency distributions of (a) fine-root mass (FRM) and (b) fine-root mass including very fine roots in RES (FRM+RES).

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LITERATURE CITED

BAKKER, M. R. 1999. Fine-root parameters as indicators of sustainability of forest ecosystems. *Forest Ecology and Management* 122:7–16.

BÖHM, W. 1979. *Methods of studying root systems*. Springer-Verlag, Berlin. 188 pp.

Table 1. Fine root mass (± SE) with and without very fine roots in RES.

	Without very fine roots in RES		With very fine roots in RES	
	Fine root mass (t ha^{-1})	Coefficient of variation (%)	Fine root mass (t ha^{-1})	Coefficient of variation (%)
Plot 1	11.8 ± 1.8 (n = 7)	40.0	18.8 ± 1.5 (n = 7)	21.2
Plot 2	10.6 ± 1.7 (n = 7)	42.1	18.0 ± 3.0 (n = 7)	43.8
Total	11.2 ± 1.2 (n = 2)	39.8	18.4 ± 1.6 (n = 2)	32.7

CAIRNS, M. A., BROWN, S., HELMER, E. H. & BAUMGARDNER, G. A. 1997. Root biomass allocation in the world's upland forest. *Oecologia* 111:1–11.

COMAS, L. H., EISSENSTAT, D. M. & LAKSO, A. N. 2000. Assessing root death and root systems dynamics in a study of grape canopy pruning. *New Phytologist* 147:171–178.

CONOVER, W. J. 1980. *Practical non-parametric statistics*. John Wiley & Sons, New York. 493 pp.

GILL, R. A. & JACKSON, R. B. 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist* 147:13–31.

JACKSON, R. B., CANADELL, J., EHLERINGER, J. R., MOONEY, H. A., SALA, O. E. & SCHULZE, E. D. 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108:389–411.

JACKSON, R. B., MOONEY, H. A. & SCHULZE, E. D. 1997. A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of National Academy of Sciences, USA* 94:7362–7366.

JARAMILLO, D. F. 1989. *Estudio general de suelos, erosión y uso potencial agropecuario para los proyectos hidroeléctricos Porce II y Porce III*. Empresas Públicas de Medellín. Medellín. 250 pp.

JOSLIN, J. D. & HENDERSON, G. S. 1984. The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride. *Forest Science* 30:965–970.

KLINGE, H. & HERRERA, R. 1978. Biomass studies in Amazon caatinga forest in southern Venezuela. 1. Standing crop of composite root mass in selected stands. *Tropical Ecology* 19:93–109.

LARA, W. 2003. *Carbono orgánico en suelos de rastrojos, bosques secundarios y bosques primarios del área de influencia del Proyecto Hidroeléctrico Porce II*. B.Sc. Thesis. Universidad Nacional de Colombia. Medellín.

POTTER, C. S. 1999. Terrestrial biomass and the effects of deforestation on the global carbon cycle. *Bioscience* 49:769–778.

SANFORD, R. L. & CUEVAS, E. 1996. Root growth and rhizosphere interactions in tropical forest. Pp. 268–300 in Mulkey, S. S., Chazdon, R. L. & Smith, A. P. (eds). *Tropical forest plant ecophysiology*. Chapman & Hall, New York.

SHESKIN, D. J. 2000. *Handbook of parametric and non-parametric statistical procedures*. Chapman & Hall, Boca Raton. 982 pp.

TRYFOS, P. 1996. *Sampling methods for applied research*. John Wiley & Sons, New York. 440 pp.