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A soil quality index based on the equilibrium between soil organic matter and biochemical properties of undisturbed coniferous forest soils of the Pacific Northwest

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ABSTRACT

Recent studies have suggested that the organic matter contents of undisturbed soils (under natural vegetation) are in equilibrium with biological and biochemical properties. Accordingly, we hypothesised that such equilibria should be disrupted when soils are subjected to disturbance or stress, and that measurement of this disruption can be expressed mathematically and used as a soil quality index. In this study, we evaluated these hypotheses in soils from the H.J. Andrews Experimental Forest in Oregon. Both O and A horizons were sampled from nine sites in Spring 2005 and Fall 2006. Soil samples were analyzed for enzyme activities (phosphatase, β-glucosidase, laccase, N-acetyl-glucosaminidase, protease and urease), and other biological and chemical properties including N-mineralization, respiration, microbial biomass C (MBC), soil organic carbon (SOC) and total nitrogen content. In addition, soil samples from one old-growth site were manipulated in the laboratory to either simulate chemical stresses (Cu addition or pH alteration) or physical disturbances (wet–dry or freeze–thaw cycles). The results showed variation in biological and biochemical soil properties that were closely correlated with SOC. Multiple regression analysis of SOC levels against all soil properties showed that a model containing only MBC and phosphatase activity could account for 97% of the SOC variation among the sites. The model fit was independent of spatial and temporal variations because covariates such as site, stand age, sampling date, and soil horizon were found to be not statistically significant. Although the application of stress/disturbance treatments inconsistently affected most of the individual biochemical properties, in contrast, the ratio of soil C predicted by the model (C_p), and soil C measured (C_m) was consistently reduced in soils submitted to at least one level of stress and disturbance treatments. In addition, C_p/C_m was more affected in soils submitted to wet–dry cycles and Cu contamination than to freeze–thaw cycles or shifts in soil pH. Our results confirm previous evidence of a biochemical balance in high quality undisturbed soils, and that this balance is disrupted when the soil is submitted to disturbances or placed under stress conditions. The C_p/C_m ratio provides a simple reference value against which the degrading effects of pollutants or management practices on soil quality can be assessed.

1. Introduction

Biochemical soil properties have been widely used as indicators of soil quality because of their essential role in soil biology, ease of measurement, and sensitivity to environmental change compared to most physical and chemical properties (Dick et al., 1996; Riffaldi et al., 2002; Miralles et al., 2007). However, the use of these parameters as indicators of soil quality, both individually and combined, has been criticized due to the lack of reference values, their contradictory behaviors when a soil is degraded, and the seasonal and regional variations in expression levels (Nannipieri, 1994; Trasar-Cepeda et al., 2000; Spedding et al., 2004; Gil-Sotres et al., 2005).

Aiming to address these limitations, Trasar-Cepeda et al. (1998) proposed a new approach to develop a biochemical-based index of soil quality that dispenses the need for reference data, and that is independent of both seasonal and among-site variations in soil conditions. They showed that in undisturbed native soils under climax vegetation there is an equilibrium between soil organic matter content and biological activity. The equilibrium was expressed by an equation that defined total soil N content as

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a function of microbial biomass C, N-mineralization capacity, and the activities of phosphatase, β-glucosidase and urease. Because soils under climax vegetation are considered to be of high quality (Fedoroff, 1987; Doran et al., 1994), and because the equation was statistically very significant ($R^2 = 0.97$, $p < 0.001$), Trasar-Cepeda et al. (1998) suggested that any disturbance or stress on a soil will modify this relationship in such a way that the total N calculated from the equation (Nc) will be either lower or higher than the total soil N content measured by Kjeldahl digestion (Nk).

Further studies demonstrated the validity of the Nc/Nk ratio to indicate soil degradation or disturbance in soils affected by management, mining, or contamination with organic effluents and with heavy metals (Leiros et al., 1999; Trasar-Cepeda et al., 2000). These studies showed that in all situations soil degradation was reflected by the Nc/Nk value, whereas none of the individual biochemical parameters responded consistently to the factors influencing soil quality. The Nc/Nk distinguished among biochemically balanced soils, soils in a transient state of high microbiological and biochemical activities (i.e., soils that had received an application of fertilizers or products that stimulate microbial activity), and truly degraded soils (Leiros et al., 1999). Finally, the approach distinguished between the effects of current pollutants versus prior soil degradation, and differentiated between pollutants in regard to their ability to cause different degrees of soil degradation (Trasar-Cepeda et al., 2000).

Despite these promising results, similar studies have not been conducted in soils elsewhere in the world other than those where the original model was developed (Galicia, Spain). Therefore, it is uncertain whether other undisturbed high quality soils also express an equilibrium between soil organic matter and biological/biochemical properties, and whether it is disrupted when these soils are degraded. In this work, we aimed to evaluate these hypotheses in soils under forest vegetation in the Western Cascade Mountains of Oregon. Multiple regression analysis was used to define a model able to predict the total soil C and N content of forest soils which had not been subjected to human intervention for at least 50 years. In analogy to the Nc/Nk ratio, we evaluated the validity of the ratio between soil C (or N) predicted by the model, and the respective soil C (or N) measured as a soil quality index. This was accomplished by accessing changes of these ratios in an undisturbed soil submitted either to chemical stress (Cu and pH), or to physical disturbance (freeze–thaw and wet–dry cycles) treatments.

2. Materials and methods

This study was conducted with soils collected from the H.J. Andrews Experimental Forest (HJA) situated in the Western Cascades of Oregon (lat. 44°15′N, long. 122°10′W) in the 6400 ha watershed of Lookout Creek, a tributary of Blue River and the McKenzie River. Mean annual temperature at the headquarters site of HJA is 8.7 °C (1973–2002) and annual precipitation during the same period is 2370 mm y^{-1}, mostly as rain (Brant et al., 2006). About 90% of the annual precipitation falls between October and April with the wettest month being December and peak drought conditions occurring in July (Franklin et al., 1990). Soils at the sampling sites were either Inceptisols formed from glacial deposits with poorly developed profiles lacking a textural B horizon and classified as either typic dystrochrepts (Carpenter series), or typic eutrochrepts (Frissel series). The other major soil type was formed in residuum and colluvium with a developed B horizon and classified as an ultic hapludalf (McKenzie River series).

Soil samples were collected during late Spring of 2005 and early Fall of 2006 from four old growth and four second growth sites. The old growth and second growth sites contained trees that were either more than 300 or approximately 50 years old, respectively. Other site properties (elevation, soil texture, pH, and vegetation cover) are presented in Table 1. At each site, soil samples were collected from four positions along the main slope gradient providing four pseudo-replications per site per season. The O horizon was sampled manually by collecting the organic material in the soil surface after removing the undecomposed litter. The A horizon was sampled in the 0–5 cm mineral soil layer using a trowel. No soil was collected from locations where ectomycorrhizal mats were visually identified. After collection, samples were refrigerated, transported in a cooler, sieved (4 and 2 mm opening for O and A horizons, respectively), and stored at 4 °C. All analyses were performed within one week of sampling.

2.1. Stress/disturbance treatments

Soil from one HJA old-growth site (site 37 – Table 1) was manipulated in the laboratory to simulate stress and disturbance. This soil was re-sampled in April 2007 by collecting a composite sample from the A horizon (0–5 cm layer). The soil was air dried, sieved through a 2 mm sieve, divided into 72 portions, each equivalent to 40 g of oven-dry soil, and placed into 150 mL flasks. This provided 64 experimental units for four treatments (with four levels of each treatment) and two sets of controls. Soil samples were pre-incubated at 25 °C and 60% of the water holding capacity (WHC) for 24 h for stress treatments, and 72 h for disturbance treatments.

Stress treatments consisted of either changing pH or increasing soil Cu concentrations, and disturbance treatments consisted of wet–dry or freeze–thaw cycles. These treatments were applied

Table 1
Characterization of sampling sites at the H.J. Andrews Experimental Forest (www.fsl.orst.edu/lter).

Site	Elev. (m)	Texture	pH	Vegetation/management history
Old growth sites				
37[b]	952	Silt loam	5.5	Douglas-fir[a] (*Pseudotsuga menziesii*); vine maple (*Acer circinatum*)
41[d]	976	Stony loam	4.5	Douglas-fir[a]; Pacific yew[a] (*Taxus brevifolia*); Pacific rhododendron (*Rhododendron macrophyllum*)
139[c]	849	Stony silty loam	5.2	Douglas-fir[a]; western red cedar (*Thuja plicata*); western hemlock (*Tsuga heterophylla*)
170[c]	784	Gravelly silty loam	4.5	Douglas-fir[a]; western red cedar; vine maple
Second growth sites				
89[c]	661	Clay loam	5.2	Douglas-fir[a]/(clearcut 1954; burned, replanted 1957)
112[b]	600	Gravelly silty clay loam	4.7	Douglas-fir[a]/(clearcut 1959; burned; natural regeneration)
113[b]	667	Silty clay loam	4.8	Douglas-fir[a]/(clearcut 1959; burned; natural regeneration)
136[c]	782	Gravelly silty loam	5.4	Douglas-fir[a]; western red cedar/(clearcut 1954; burned; replanted 1967)

[a] Dominant species on each site.
[b] Soil types on the site McKenzie River series, Ultic Hapludalf.
[c] Soil types on the site Carpenter series, Typic Dystrochrept.
[d] Soil types on the site Frissell series, Typic Eutrochrept.

according to Degens et al. (2001) with some modifications. For pH stress, the current soil pH (5.5) was increased or decreased by one or two pH units (3.5, 4.5, 6.5, and 7.5) using solutions of NaOH (0.5 M) or HCl (1 M), respectively. The amounts of NaOH and HCl required to attain each pH level were determined from pH–buffer curves. Different levels of Cu were attained by applying $CuSO_4$ at rates of 50, 200, 800 and 3200 μg Cu g^{-1} soil. Decreases in soil pH caused by $CuSO_4$ additions were corrected by adding sufficient NaOH to adjust the pH back to its initial level.

Disturbance simulated by wet–dry cycles consisted of air-drying soil with fan-forced air at 20–25 °C for 24 h, followed by rapid rewetting to 60% WHC and incubation for 24 h in the dark. Soil samples were subjected to one, two, four, or eight successive wet–dry cycles. A separate set of soils was subjected to one, two, four, or eight successive freeze–thaw cycles. Each freeze–thaw cycle consisted of freezing at −20 °C for 24 h followed by thawing and incubation at 25 °C and 60% WHC for 24 h.

Following application of both pH and Cu treatments, soils were incubated at 25 °C and 60% WHC for 4 weeks. For the two disturbance treatments, soils were incubated for 15 d counted from the last drying or freeze cycle. This time interval was considered adequate to allow sufficient re-equilibration of the microbial communities after imposition of each disturbance or stress treatment and for decomposition of organic C released from microorganisms killed by the treatments (Degens et al., 2001). Each treatment was applied to four replicates. The two sets of controls (one for each group of stress and disturbance treatments) were continuously incubated at 25 °C and 60% WHC.

2.2. Soil analyses

Gravimetric water content was determined after drying samples at 54 °C for 36 h. pH was determined on a pH meter after equilibrating 10 g of wet soil with 30 mL of deionized water for 60 min. Soil organic carbon (SOC) and total N were determined by dry combustion with an LECO CNS-200 analyzer (LECO Corp., St. Joseph, MI).

2.3. Soil enzymes

Enzyme assays were performed by slurrying 10 g of wet soil in 30 mL of deionized water. The resulting slurries were continuously stirred on a magnetic stir plate while 1 mL aliquots were dispensed into test tubes (2 replicates per sample per assay). Activities of β-glucosidase, phosphatase and N-acetyl-glucosaminidase were measured by conventional *p*-nitrophenyl-ester based assays (Tabatabai, 1994; Parham and Deng, 2000), with some modifications proposed by Caldwell et al. (1999). One mL aliquots of soil slurry were incubated at 30 °C with 1 mL of either 10 mM *p*-nitrophenyl-β-glucoside, 50 mM *p*-nitrophenyl-phosphate or 10 mM *p*-nitrophenol-N-acetylglucosamide. The phosphatase activity was run unbuffered to measure enzyme activity at soil pH (Caldwell et al., 1999). After 1 h, 0.5 mL aliquots of 0.5 M $CaCl_2$ were added and reactions terminated by adding either 2 mL of 0.1 M pH 12 tris(hydroxymethyl)aminomethane to the β-glucosidase assay, or 2 mL of 0.5 M NaOH to the phosphatase and N-acetyl-glucosaminidase assays. Controls consisted of slurry without substrate and substrate without slurry.

Phenol oxidase activity was measured spectrophotometrically by adding to each soil slurry 1 mL of 5 mM L-3,4-dihydroxyphenylalanine (DOPA) in 50 mM acetate buffer, pH 5 (Sinsabaugh et al., 1999). Controls consisted of adding 1 mL of acetate buffer to soil slurries. After 1 h of incubation at 30 °C, 1 mL of 0.6% (w/v) sodium azide solution was added to stop the reaction. The suspensions were centrifuged and the activity quantified by measuring absorbance at 460 nm. Calculation of the quantity of dihydroindole–quinone–carboxylate formed was based on a micromolar extinction coefficient of 1.6 (Sinsabaugh et al., 1999).

Protease activity was determined as the rate of tyrosine equivalents released from casein substrate according to Ladd and Butler (1972) with the following modifications. One mL of soil slurry was added to 1 mL of caseinate (0.2 g l^{-1}) + sodium azide (0.1 g l^{-1}) solution and incubated at 30 °C. After 24 h, the reaction was stopped with 1 mL of trichloroacetic acid. The concentration of tyrosine equivalents in 1 mL aliquots of supernatant was determined colorimetrically (578 nm) using the Lowry method. Controls consisted of substrate solution mixed with 1 mL of dH_2O instead of soil slurry. Activity was expressed as μg of tyrosine equivalents released per gram of dry soil per hour.

Urease activity was measured by determination of NH_4^+ released after the incubation of soil samples with a buffered urea solution for 2 h at 37 °C (Kandeler and Gerber, 1988). Ammonium was determined with an Astoria-Pacific series 300 autoanalyzer (Astoria-Pacific, Inc., Clackamas, Oreg.).

2.4. Soil respiration and net N-mineralization

Soil respiration was determined by adding portions of soil (30 and 15 g for A and O horizons, respectively), with water contents adjusted to 70% of WHC, to 500 mL Mason jars. The jars were sealed with caps containing a rubber septum and incubated at 25 °C for 20 d. At 5-d intervals, the air in the overhead space was sampled from each jar and CO_2 determined by chromatography. After each reading the jars were left open for half an hour to ventilate and then re-sealed.

The rate of net N-mineralization was determined by measuring the difference in inorganic nitrogen levels in soil samples before and after 20 d of incubation. The soil samples were the same as used to measure soil respiration. Extractions were performed on 5 g portions of wet soil with 15 mL of 2 M KCl. Aliquots of the filtrate were analyzed for NH_4^+ and NO_3^- with an autoanalyzer. Nitrogen mineralization was expressed as μg of mineral N produced per gram of dry soil per day.

2.5. Microbial biomass C

Microbial biomass C (MBC) was determined by the chloroform fumigation extraction method, using 0.5 M K_2SO_4 as extractant (Vance et al., 1987). The organic C of extracts was estimated by combustion in a mass spectrometer. The difference in C content between the fumigated and unfumigated extracts was converted to MBC by applying a factor (K_c) of 0.45 (Jenkinson, 1988). Results were expressed as mg of MBC per gram of dry soil. The metabolic quotient, qCO_2 (microbial respiration per unit of biomass), was calculated and expressed in μg CO_2-C mg^{-1} biomass C d^{-1} (Anderson and Domsch, 1985).

All chemical, biochemical and biological soil properties described above were analyzed in soil samples collected during Spring 2005. Analyses of this data set revealed the best predictors of SOC and total N (details in the "Statistical analyses" section). Therefore, besides SOC and total N, all samples collected afterwards were analyzed only for phosphatase, β-glucosidase, phenol oxidase, respiration and MBC. These included samples from Fall 2006 and those subjected to stress and disturbance treatments.

2.6. Statistical analyses

Analytical results obtained from site pseudo-replicates within each sampling date were pooled before the statistical analysis.

Multiple linear regression was used to define equations (models) able to predict SOC and total N based on the chemical and biochemical soil properties analyzed. All variables were subjected to natural logarithmic transformation (except N-mineralization, which included negative values) in order to assure homoscedasticity and linearity between the dependent and the explanatory variables. A preliminary model was selected using the Spring 2005 data set which included a total of 10 explanatory variables. The Mallows' C_p statistic (Mallows, 1973) was used to provide the best subset selection of predictors by examination of all possible regressions. The best model was considered that with the smallest number of parameters with acceptable "*p*-values", and a Mallows' C_p value closest to the number of parameters. Alternative models were also selected after removing each one of the previously selected predictors from the data set. For example, if the variables "A" and "B" were selected using the original data set, two alternative models were defined using the same pool of original variables but excluded either "A" or "B". The selected predictors in both main and alternative models were the ones analyzed in soil samples collected in the Fall 2006 and in the soils submitted to stress and disturbances.

A second data set which included Fall 2006 samples was used to evaluate the consistency of the predictors previously selected. The definition of a final consensus model was performed by combining both the Spring 2005 and Fall 2006 data sets. The robustness of all the selected models to spatial variation was evaluated by testing the statistical significance of site, stand age and soil horizon when these were added as covariates. Temporal robustness was evaluated in the consensus model by testing the statistical significance of "season" as covariate. Multi-collinearity among the model predictors was evaluated by the variance inflation factor (VIF) (Marquard, 1970) and using 10 as cut-off value.

A two-sided multiple comparison with a control (MCC) test was used to evaluate the effects of stresses and disturbances on soil biochemical properties, and on the ratio between the SOC predicted by the model (C_p) and the SOC measured (C_m). All statistical analyses were performed using S-Plus 8.0 (Insightful Corp.).

3. Results

3.1. Variation in soil properties

Total SOC measured among the sites ranged from 3.4 to 13.4% in the 0–5 cm of the A horizon, and from 17.5 to 39.6% in the O horizon (Table 2). Soil total N values ranged from 0.12 to 0.30% in the A horizon, and from 0.27 to 1.13% in the O horizon. Variations in soil biochemical properties within soil horizon were generally in the same order of magnitude of that for SOC and total N. For instance, the coefficient of variation (CV) for SOC in both soil horizons ranged from 23.8% in the O horizon to 36.3% in the A horizon, whereas the CV for all biochemical properties (except for the rate of N-mineralization) ranged between 17 and 42%. Generally, there was a large difference in soil properties between the A and O horizons. Nevertheless, the magnitude of this difference was dependent on the soil property considered. The O/A ratio was 4.7 for SOC and 3.3 for total N, and ranged from 2.6 to 5.7 for the soil enzymes analyzed. The lowest O/A ratio calculated was for N-mineralization (−0.6) while the highest ratios were for soil respiration activity and MBC, which were equivalent to 9.9 and 9.3, respectively (Table 2).

Few soil properties differed when comparing old growth and second growth sites. Except for respiration, which was 23% higher in both O and A horizons of old growth sites ($p < 0.05$), the observed differences occurred only when comparing the O horizons. Accordingly, the O horizon of old growth sites had higher values of SOC (37%; $p < 0.01$), total N (102%; $p < 0.001$), and phosphatase (58%; $p < 0.05$) in relation to second growth sites. A similar trend was observed for MBC (34%) and for β-glucosidase (37%), but at a lower statistical significance level ($p < 0.10$). No significant differences regarding stand age were found for phenol oxidase, protease, N-acetyl-glucosaminidase, urease, and N-mineralization either in O or A soil horizon.

As might be expected, biological soil properties showed higher seasonal fluctuations than SOC and total N (Table 3). In general, soil samples collected during Fall 2006 had lower microbial activities (respiration, enzyme activity and qCO_2) compared to Spring 2005; yet their MBC values were either similar (O horizon) or higher (A horizon) than those observed during the Spring. The highest seasonal fluctuations in the A horizon occurred in MBC, qCO_2, and phosphatase activity, whereas the same was valid for soil respiration and qCO_2 in the O horizon.

3.2. Correlation between SOC, total N, and biochemical properties

Most of the biological properties were highly correlated with SOC (Table 4). The highest significant correlation coefficients were obtained for phosphatase activity ($r = 0.978$), MBC and soil respiration (both with $r = 0.971$), whereas the lowest was for urease activity ($r = 0.758$). Only N-mineralization and qCO_2 were not significantly correlated with SOC. Correlation of soil total N with biological properties was slightly lower compared to those obtained for SOC. The highest correlation was for phosphatase ($r = 0.904$) following by phenol oxidase ($r = 0.877$), MBC ($r = 0.873$), β-glucosidase ($r = 0.857$), and respiration ($r = 0.850$). Similar to SOC, the lowest significant correlation was for urease

Table 2
Statistical descriptions of values of chemical and biochemical properties of the H.J. Andrews Forest soils (Table 1).

	O horizon				A horizon (0–5 cm)				O/A horizon[a]
	Min	Max	Mean	CV (%)	Min	Max	Mean	CV (%)	
Soil organic C (%)	17.5	39.6	29.9	24	3.4	13.4	6.3	36	4.7
Total N (%)	0.27	1.13	0.69	39	0.12	0.30	0.21	24	3.3
β-Glucosidase (μmol PNP g^{-1} h^{-1})	9.8	33	17	36	1.9	6.5	3.8	37	4.5
Phosphatase (μmol PNP g^{-1} h^{-1})	81	319	173	36	23	84	41	39	4.2
Glucosaminidase (μmol PNP g^{-1} h^{-1})	11	25	20	24	4.3	7.4	5.8	18	3.4
Phenol oxidase (mmol diqc g^{-1} h^{-1})	117	313	199	34	25	86	57	35	3.5
Urease (μg NH_4^+-N g^{-1} h^{-1})	72	260	176	38	50	86	67	17	2.6
Protease (μg Tyr g^{-1} h^{-1})	1.5	3.6	2.5	30	0.1	0.7	0.4	41	5.7
N-mineralization (μg N g^{-1} d^{-1})	−0.8	0.5	−0.1	429	0.0	0.5	0.2	97	−0.6
Soil respiration (μg CO_2-C g^{-1} d^{-1})	115	513	346	35	23	54	35	26	9.9
Microbial biomass C (mg C g^{-1})	2.3	6.9	4.8	24	0.3	0.9	0.5	26	9.3
qCO_2 (μg CO_2-C mg^{-1} biomass C d^{-1})	2.2	6.0	3.2	34	1.6	6.0	3.4	42	1.06

[a] Ratio between average values of O and A horizons.

Table 3
Seasonal differences in chemical and biochemical soil properties expressed as a percentage of change that occurred in Fall 2006 relative to Spring 2005.

	% Change	
	O horizon	A horizon
Soil organic C	−5	10
Total N	14	−6
β-Glucosidase	−16	−20
Phosphatase	5	−28
Phenol oxidase	−11	−10
Respiration	−34	−16
Microbial biomass C	−2	44
qCO_2	−37	−42

($r = 0.539$), and N-mineralization and qCO_2 were not correlated with total N.

3.3. Selection of SOC and total N predictors

The analysis of the Spring 2005 data set showed that phosphatase activity and MBC were the most reliable SOC predictors based on the Mallows' C_p statistic analysis (Table 5). The equation fitted through multiple linear regression using these two variables was able to explain 98% of the SOC variation of the sites. Alternative sets of predictors for SOC were also evaluated after omitting either phosphatase or MBC from the original data sets (Table 5). This analysis selected soil respiration and phenol oxidase activity as substitutes for MBC and phosphatase, respectively. Nevertheless, these alternative data sets presented limitations associated with high multi-collinearity among predictors (VIF > 10), or spatial dependency as indicated by significant statistical p-values for one of the tested covariates (Table 5).

The consistency of phosphatase and MBC as predictors of SOC was confirmed when using the second data set obtained in the Fall 2006. Similarly to the model obtained with the Spring 2005 data, phosphatase and MBC were able to explain 98.3% of the SOC variability of samples collected in Fall 2006. The consensus model, adjusted using the combination of both Spring 2005 and Fall 2006 data sets, was able to explain 97% of the SOC variation using the following equation:

$$\ln(\text{SOC}) = 1.236 + 0.276\ln(\text{phosphatase}) + 0.289\ln(\text{MBC}) \quad (1)$$

where total C is expressed as percentage, phosphatase activity in μmol of PNP g^{-1} soil h^{-1} and MBC in mg C-biomass g^{-1} soil. This model was shown to be robust to seasonal and spatial variations because none of the tested covariates (season, site, stand age and soil horizon) were statistically significant when added to the model above. In addition, the relative low value of VIF (5.6) indicated an acceptable level of multi-collinearity among the two predictors.

Table 4
Correlation coefficients between SOC, total N, and biological and biochemical soil properties.

Soil property	SOC	Total N
Phosphatase	0.978***	0.904***
Microbial biomass C	0.971***	0.873***
Respiration	0.971***	0.850***
N-acetyl-glucosaminidase	0.942***	0.766***
Phenol oxidase	0.935***	0.877***
Protease	0.915***	0.751***
β-Glucosidase	0.894***	0.857***
Urease	0.758***	0.539*
N-mineralization	−0.341	−0.171
qCO_2	−0.064	−0.072

*$p < 0.05$; ***$p < 0.001$.

Several sets of predictors of total N were also selected using the same criteria used for SOC (Table 5). Nevertheless, the coefficients of determination (R^2) for all fitted models were lower than those obtained for SOC, and most models presented either elevated VIF values, or significant spatial or temporal covariates. For these reasons, a suitable equation relating total soil N with soil biochemical properties could not be defined.

3.4. Effect of stress and disturbance on biochemical soil properties

Stress and disturbance treatments simulated on soil samples from site 37 caused different effects on the biological and biochemical properties. The application of 1, 2, 4 or 8 freeze–thaw cycles significantly reduced β-glucosidase activity and MBC (Table 6). However, changes in β-glucosidase were not proportional to the disturbance levels as occurred for MBC. Phosphatase, phenol oxidase, respiration, and qCO_2 activities did not respond to freeze and thaw cycles. When disturbances were applied as wet–dry cycles, MBC was significantly reduced by all treatment levels, whereas the activities of the three enzymes were only affected after four or more wet–dry cycles. This treatment did not cause significant change to soil respiration or qCO_2 (Table 6).

Although addition of Cu or modification of soil pH affected all five biochemical soil properties (Table 6), there was either no consistency in the change in response to the stress, or it was in the opposite direction from that observed after disturbance treatments. For example, soil respiration activity was increased by the two lowest Cu doses, and decreased by the two highest ones. Moreover, qCO_2, β-glucosidase and phenol oxidase activities tended to increase with the increase of Cu added to the soil, whereas MBC and phosphatase activity decreased. Increase of soil pH from 5.5 to 6.5 or 7.5 also increased the phenol oxidase activity in addition to soil respiration and MBC. However, a shift from 5.5 to pH 4.5 or 3.5 significantly increased the activities of all three soil enzymes — especially of phenol oxidase and β-glucosidase — and decreased soil respiration, MBC and qCO_2.

3.5. Changes in the C_p/C_m ratio

The ratio between soil C predicted by equation (1) and soil C actually measured (C_p/C_m) varied consistently with the levels of stress and disturbances. The C_p/C_m was close to unity in the two sets of controls, and tended to decrease with the increase of the stress or disturbance applied (Fig. 1). In all disturbance and stress treatments there was no significant change in SOC relative to controls after the incubation period. Therefore, the decreases observed in C_p/C_m resulted exclusively from an underestimation of the predicted C (C_p).

Application of 4 and 8 freeze–thaw cycles significantly decreased C_p/C_m from 0.99 in the control to 0.93 and 0.92, respectively (Fig. 1A). Wet–dry cycles caused a more drastic effect on soil, decreasing C_p/C_m from 0.93 after 1 freeze–thaw cycle to 0.85 after 8 cycles (Fig. 1B). Soils treated with Cu also showed a significant decrease in C_p/C_m, but only at the two highest doses applied (800 or 3200 μg g^{-1} Cu). Notwithstanding, the application of the highest dose changed C_p/C_m to 0.68, the lowest value among all stress and disturbance simulations (Fig. 1D). Increase in soil pH did not produce changes in C_p/C_m; however, a significant decrease in this ratio was observed when the soil pH was shifted to 3.5 (Fig. 1C).

4. Discussion

The high degree of variability of soil biological and biochemical properties due to climate, season, geographical location, and pedogenetic factors has been cited as the main problem that

Table 5
Best set of explanatory variables for SOC and total N content defined by multiple regression analysis using the Spring 2005 (*S*) data set.

Dependent variable	Data set	Selected explanatory variables	R^2	C_p[a]	VIF[b]	df	Significant covariates
SOC	*S*	PME***; MBC**	0.979	7.6	8.9	13	
SOC	*S* without MBC	PME**; RESP*	0.972	5.1	11	13	
SOC	*S* without PME	MBC***; PhOx*	0.959	5.0	4.1	13	Stand age*
Total N	*S*	PME***; NAG*	0.881	2.2	10	13	
Total N	*S* without NAG	PME***; RESP*	0.837	−1.0	11	13	Stand age*
Total N	*S* without PME	β-GLC***	0.645	−1.7	ND	14	Stand age*

PME, phosphatase; MBC, microbial biomass C; RESP, respiration; PhOx, phenol oxidase; NAG, N-acetyl-glucosaminidase; β-GLC, β-glucosidase.
***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.
[a] Mallows' C_p statistic (Mallows, 1973).
[b] Variance inflation factor (Marquard, 1970).

severely limits their use as soil quality indicators (Gil-Sotres et al., 2005). Such high spatial and temporal variations were demonstrated in this and several other studies (Trasar-Cepeda et al., 1998; Leiros et al., 2000; Chen et al., 2003; Miralles et al., 2007). Notwithstanding, most biological and biochemical soil properties were highly correlated with SOC, and to a lesser degree with total N, despite differences in soil horizon, season, and sampled sites. Significant correlation coefficients between SOC and soil biochemical properties have been described in other forest and agricultural soils (Perucci et al., 1997; Trasar-Cepeda et al., 1998; Leiros et al., 2000; Chodak et al., 2003; Nsabimana et al., 2004; Miralles et al., 2007), but values as high as the ones found in this study have been rarely reported (e.g., Haynes, 1999). One explanation for these high correlation values may be the wide range of values obtained for SOC and most biochemical properties analyzed (Table 2).

In fact, SOC plays an important role in determining the size of the microbial biomass and the level of soil enzyme activity. Higher contents of SOC can support more biological activity because the energy and available nutrient status in soils are enhanced (Nourbakhsh, 2007). Our results demonstrated that such a relationship between SOC and biological and biochemical properties is in equilibrium in high quality soils (undisturbed native soils) as previously suggested by Trasar-Cepeda et al. (1998). This equilibrium was expressed in a spatially and temporally robust model that could explain 97% of the SOC variation in the HJA forest soils using only MBC and phosphatase activity as predictors (Table 5; Eq. (1)). The adjustment of this model was as high as that obtained by Trasar-Cepeda et al. (1998) to express the total soil N of undisturbed soils in Galicia, Spain. Interestingly, their model required five biological and biochemical properties as predictors, and also included MBC and phosphatase activity. This fact confirms the close association of these two properties with SOC in undisturbed soils of different ecosystems.

Trasar-Cepeda et al. (1998) suggested that the equilibrium between total N and biochemical properties expressed in their model would be disrupted by variations in the biochemical quality of soils as a result of degradation processes. Subsequent studies showed that in agricultural and polluted soils the ratio between the calculated total soil N (Nc) using the equation previously defined, and the total N obtained by Kjeldahl method (Nk) was consistently altered relative to undisturbed soils (Leiros et al., 1999; Trasar-Cepeda et al., 2000; Miguéns et al., 2007). Therefore, the Nc/Nk ratio was proposed as an index of the biochemical quality of soils. By analogy, we tested the validity of the ratio between soil C

Table 6
Effect of different disturbance and stress treatments on individual soil biological and biochemical properties. Control values are in bold.

	β-Glucosidase ($\mu mol\ g^{-1}\ h^{-1}$)	Phosphatase ($\mu mol\ g^{-1}\ h^{-1}$)	Phenol oxidase ($mmol\ g^{-1}\ h^{-1}$)	Respiration ($\mu g\ CO_2\text{-}C\ g^{-1}\ d^{-1}$)	MBC ($mg\ C\ g^{-1}$)	qCO_2 ($\mu g\ CO_2\text{-}C\ mg^{-1}\ MBC\ d^{-1}$)
Freeze–thaw cycles						
0	**1.99**	**33.4**	**0.13**	**19.1**	**0.36**	**0.53**
1	1.60*	35.6	0.13	15.5	0.33*	0.47
2	1.66*	35.1	0.13	16.4	0.32*	0.52
4	1.39*	31.6	0.12	16.3	0.30*	0.55
8	1.67*	34.3	0.13	15.9	0.27*	0.59
Wet–dry cycles						
0	**1.99**	**33.4**	**0.13**	**19.1**	**0.36**	**0.53**
1	1.90	31.3	0.12	17.8	0.30*	0.52
2	1.89	30.6	0.12	19.2	0.31*	0.61
4	1.62*	27.3*	0.11*	19.3	0.28*	0.68
8	1.54*	26.5*	0.10*	15.2	0.26*	0.58
Cu added ($\mu g\ g^{-1}$)						
0	**1.61**	**30.3**	**0.11**	**14.7**	**0.41**	**0.36**
50	1.85	30.0	0.13*	16.9*	0.39	0.43
200	2.03	29.3	0.14*	16.2*	0.38	0.43
800	1.74	22.9*	0.20*	13.5*	0.31*	0.44
3200	3.52*	14.0*	0.35*	13.3*	0.22*	0.61*
Adjusted pH						
7.5	1.39	27.8	0.20*	18.2*	0.46*	0.40
6.5	1.69	32.1	0.16*	16.6	0.42	0.40
5.5 (control)	**1.61**	**30.3**	**0.11**	**14.7**	**0.41**	**0.36**
4.5	1.81	31.1	0.18*	10.8*	0.37*	0.29
3.5	2.79*	34.1*	0.20*	7.1*	0.30*	0.24*

*Values are statistically different from the control ($p < 0.05$) within each soil property and stress/disturbance treatment.

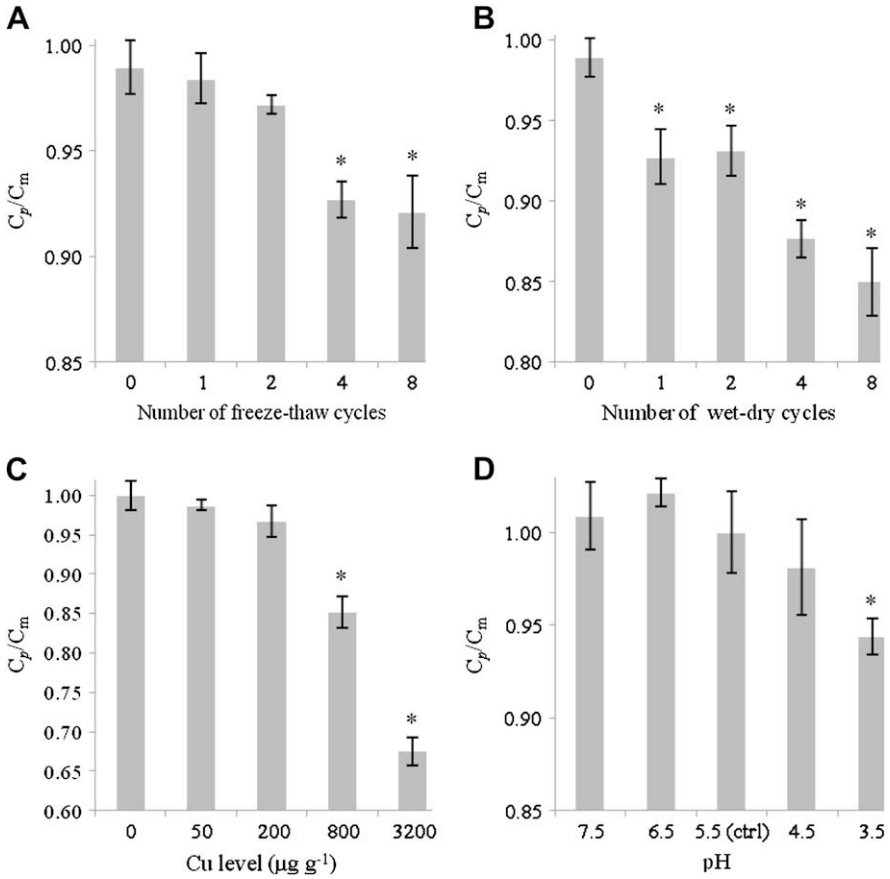

Fig. 1. Changes in the ratio between the SOC predicted using equation (1) and SOC measured (C_p/C_m) in soils samples subjected to increasing levels of disturbance/stress imposed by freeze–thaw (A); by wet–dry cycles (B); by Cu addition (C); by changing soil pH (D). Error bars indicate the standard error of means (4 replicates). Asterisks indicate significant difference from respective controls ($p < 0.05$).

predicted by the equation (1) (C_p), and soil C measured (C_m) to indicate the degradation caused by disturbances and stresses applied to one of the HJA forest soils. The ability of both C_p/C_m and individual biochemical properties to respond to these treatments is discussed in the following sections.

4.1. Response of individual soil properties to stresses and disturbances

A suitable soil quality indicator must be: (a) sensitive to as many degrading agents as possible; (b) show a consistent directional change in response to a given contaminant, and (c) be able to reflect different levels of degradation (Elliott, 1997). Most of the biological and biochemical properties analyzed in soils submitted to stresses and disturbances failed in at least one of these requirements (Table 6). For instance, β-glucosidase, phosphatase, phenol oxidase, respiration and qCO_2 activities were insensitive or unable to differentiate the level of freeze–thaw disturbance. Additionally, the decrease of qCO_2 with soil acidification was contrary to the trend observed in another study (Blagodatskaya and Anderson, 1999). Soil enzymes also expressed complex responses to the stress treatments. For example, β-glucosidase and phosphatase activities increased when soil pH was lowered, but they responded in opposite directions to addition of Cu with β-glucosidase being stimulated while phosphatase was inhibited. An increase of β-glucosidase activity in response to Cu was also observed in soils incubated in the laboratory with up to 10,000 μg g^{-1} of Cu for 4 weeks (Leiros et al., 1999). In contrast, heavy metal contaminated sites (Cd, Zn, Cu and Pb) showed reduced activity of this enzyme compared to uncontaminated ones (Lee et al., 2002). Complex and inconsistent behavior of soil enzymes has also been described in soils polluted by different types of contaminants (Trasar-Cepeda et al., 2000; Gianfreda et al., 2005).

Microbial biomass C was the only variable that changed consistently in response to all stress and disturbance treatments. MBC was also considered to be the most reliable biochemical parameter by 41% of authors on a list of papers where individual soil properties were studied as soil quality indicators (Gil-Sotres et al., 2005). Nevertheless, this review also describes a series of results showing contradictory responses of MBC to soil management or contamination. In another study, MBC was not affected or slightly increased in a cropped and a pasture soil submitted to similar pH and Cu stresses or wet–dry and freeze–thaw disturbances (Degens et al., 2001). The validity of MBC as a reliable indicator of soil quality can also be questioned when spatial and seasonal variabilities in natural and agricultural soils are considered (Murphy et al., 1998; Corre et al., 2002; Chen et al., 2003). This fact precludes the definition of reference values for MBC, which are necessary to access the magnitude of soil degradation relative to its previous state.

4.2. Validity and sensitivity of C_p/C_m as a soil quality index

The ratio C_p/C_m was consistently reduced by the increasing levels of stress and disturbances applied to the soils (Fig. 1). In addition, C_p/C_m was able to discriminate among the disturbance/stress treatments. For example, soils treated with wet–dry cycles and Cu seemed to be more affected than those treated with freeze–thaw cycles and shifts in pH (Fig. 1). Wet–dry cycles also caused

greater declines in microbial catabolic evenness of a cropped soil compared to freeze–thaw cycles (Degens et al., 2001). However, this microbial parameter underwent a greater decline in response to soil acidification than additions of up to 1000 μg Cu g^{-1} soil. The negligible alteration in C_p/C_m in response to increase in soil pH from 5.5 to 7.5 suggests that this treatment did not alter the soil biochemical balance. Such a response could be expected if the greater negative effects of soil acidification on a microbial community are considered relative to neutral conditions (Blagodatskaya and Anderson, 1999). Clearly, in our study, the C_p/C_m response to a physical or chemical disturbance was most pronounced when both biomass and phosphatase activity declined (wet–dry cycles and Cu treatments, Fig. 1B and C). However, situations were also identified where the microbial community responded to a disturbance with a combination of lower biomass and either higher phosphatase activity (acidification to pH 3.5) or no change in activity (freeze–thaw cycles). In these latter situations the responses of C_p/C_m to treatments were less pronounced (Fig. 1A and D). Further work is needed to address the longer term impact of these different types of response on soil quality, and to address the issue of resistance of the adapted community biomass and its associated activities to further stress.

Considering our results, C_p/C_m ratio can be considered a reliable biochemically-based soil quality index. This index can be obtained directly by equation (1) rewritten as:

$$\text{Index}(C_p/C_m) = (17.12/C_m)^{0.435}(\text{phosphatase}/C_m)^{0.276} \times (\text{MBC}/C_m)^{0.289}$$

where C_m (SOC measured) is expressed as a percentage, phosphatase activity in μmol of PNP g^{-1} soil h^{-1} and MBC in mg C-biomass g^{-1} soil. In contrast to individual biological and biochemical soil properties, the C_p/C_m provides a simple reference value against which the degrading effects of pollutants or management practices on soil quality can be readily accessed. Therefore, C_p/C_m will be 1 in high quality, undisturbed soils (where the ratios of MBC to SOC and phosphatase to SOC are fairly constant) and will increase or decrease according to the intensity of soil degradation. On a cautionary note, we could not fit a similar equation to predict the soil total N of the H.J. Andrews Forest soils using the same set of variables selected by Trasar-Cepeda et al. (1998). In our study we noted that net N-mineralization did not correlate with either SOC or total N, whereas in the Spanish study of Trasar-Cepeda et al. (1998) good correlations were obtained. It is entirely possible that the quality and availability of N differ between the two forest types, and that the tempo of N cycling involves different biological strategies and drivers. In addition, the fit of their equation to our data set resulted in highly overestimated values of soil total N. Therefore, it is likely that the specific model describing the C_p/C_m ratio might only be valid for the range of soils and plant ecosystems on which the model was developed as originally suggested by Gil-Sotres et al. (2005). As a final point, it should be noted that the validity and consistency of the C_p/C_m response were tested under a limited range of stresses and disturbances under laboratory conditions. Although it is likely that similar responses will occur under field conditions or as a result of other degrading agents (Leiros et al., 1999; Trasar-Cepeda et al., 2000; Miguéns et al., 2007), further studies should be carried out to evaluate the response of C_p/C_m to soil degradation under field conditions.

5. Conclusion

This study is only the second report in the soil's literature providing evidence for a biochemical balance in high quality undisturbed soils, which can be expressed by a simple mathematical model. In addition, our results confirm the hypothesis that such balance is disrupted when the soil is subjected to disturbances or is under stress conditions, as previously observed in other soils. On a cautionary note, however, the two models developed by us and by Spanish workers were not complimentary, suggesting that more work is needed to delineate the boundaries of validity of different models. Keeping in mind the limitations presented above, the use of the C_p/C_m ratio as a soil quality index remains compelling because it provides a simple and straightforward interpretation of the status of soil degradation, and requires analysis of only a few soil properties.

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