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Chemical Characterisation of a Standard Set of Organic Materials

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Abstract

This paper reports on the chemical characterisation of a standard sample set prepared for a cross-methods analysis to identify potential proximate analysis methods for the parameterisation of simulation models. In the subsequent sections, these samples are analysed using aerobic incubations, in vitro dry matter digestibility and near infrared reflectance spectrometry. Thirty-two organic materials were collected from various locations in Kenya, comprising plant samples (leaves, stems, leaflets), sawdust and cattle manure. Data obtained from chemical analysis of the materials were used to group the materials into the following quality classes: Class I, %N >2.5%, lignin <15% and soluble polyphenols <4%; Class II, %N > 2.5%, lignin >15% and soluble polyphenol >4%; Class III % N <2.5%, lignin <15%; and Class IV %N <2.5%, lignin >15%. Results showed that materials high in %N were also high in other nutrients (P, Ca, Mg), but potassium was not correlated with N concentration. Class I materials were mainly leaves of leguminous species. Class II comprise mainly *Calliandra calothyrsus* from different locations, as they had polyphenol contents higher than the critical value of 4% and had high protein-binding capacities. They were also low in K concentration (< 1%). Materials in quality class II were subdivided into three categories depending on their polyphenol and lignin contents. Class III materials were crop residues (except for one sample) and were generally low in N, polyphenols and lignin, while class IV (low in N and high in lignin) comprise stems and leaf-litter materials.

Organic materials play a critical role in both short-term nutrient availability and longer-term maintenance of soil organic matter in most smallholder farming systems in the tropics. Over the last decade, the formulation of research hypotheses related to residue quality and N release has led to a vast amount of research aimed at validation of these hypotheses. Based on much of this work, a minimum data set of resource quality parameters has been proposed for the purpose of identifying robust plant quality indices that provide improved prediction of decomposition, nutrient release and soil organic matter factors which can be coupled with decomposition models (Palm and Rowland 1997).

Plant materials containing at least 2.5% N are usually described as being of high quality, where the application of these materials to soil is likely to result in net release of nitrogen if lignin and polyphenol are <15% and <4%, respectively. On the other hand, plant materials containing less than 2.5% N are considered to be of low quality as they are likely to temporarily immobilise N during decomposition (Palm et al. 2001).

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Representation of these quality parameters in simulation modelling is limited, with C:N and lignin content the only parameters presently used by most models. Also, lack of standardisation of analysis has to date not identified robust and cheap methods that can be used to generate the data required for simulation model parameterisation. Therefore, 32 organic materials commonly used in soil fertility management in Kenya, and that covered the four resource categories of Palm et al. (2001) (see Figure 1 of Vanlauwe and Sanginga (2004)), were collected (Table 1) and characterised as an initial step in the process of describing their nutrient supply characteristics.

This paper reports on the proximate analysis conducted by the Tropical Soil Biology and Fertility Institute of CIAT and the analyses of protein-binding capacity conducted by Imperial College at Wye.

Materials and Methods

Total nutrient analysis

Materials were oven dried at 30–35°C and ground to pass through 1 mm sieve. Plant nutrients (N, P, K, Ca and Mg) were analysed through complete oxidation of 0.3 g of material by Kjedahl digestion using

Table 1. Organic materials and their place of collection.

	Sample name	Place of collection
1	*Zea mays* stover	W. Kenya
2	*Croton megalorapus* leaves	W. Kenya
3	*Senna spectabilis* leaflets	W. Kenya
4	*Lantana camara* leaves	W. Kenya
5	*Calliandra calothyrsus* leaflets	W. Kenya
6	*Senna siamea* leaflets	W. Kenya
7	*Crotalaria ochroleuca* leaflets	W. Kenya
8	*Crotalaria grahamiana* leaflets	W. Kenya
9	*Tithonia diversifolia* leaves	W. Kenya
10	*Gliricidia sepium* leaflets	W. Kenya
11	*Gliricidia sepium* leaflets	Machakos
12	*Senna siamea* leaflets	Machakos
13	*Flemingia congesta* leaflets	Machakos
14	*Senna spectabilis* leaflets	Machakos
15	*Calliandra calothyrsus* leaves	KARI-Embu (Embu provenance)
16	*Calliandra calothyrsus* leaflets	KARI-Embu (Embu provenance)
17	*Calliandra calothyrsus* leaves	KARI-Embu (Patalul provenance)
18	*Calliandra calothyrsus* leaflets	KARI-Embu (Patalul provenance)
19	*Calliandra calothyrsus* leaves	KARI-Embu (San Ramon Provenance)
20	*Calliandra calothyrsus* leaflets	KARI-Embu (San Ramon Provenance)
21	*Saccharum officinarum* stover	Nairobi
22	*Lantana camara* leaves	Nairobi
23	*Lantana camara* stems	Nairobi
24	Cattle manure	W. Kenya
25	*Tithonia diversifolia* leaves	Nairobi
26	*Gliricidia sepium* stems	Muguga
27	*Senna spectabilis* leaves	Muguga
28	*Sesbania sesban* leaves	Muguga
29	*Gliricidia sepium* leaflets	Muguga
30	*Sesbania sesban* stems	Muguga
31	*Eucalyptus saligna* leaf litter	Muguga
32	Sawdust	Muguga

sulfuric acid, hydrogen peroxide and selenium digestion mixture (Anderson and Ingram 1993). Nitrogen and potassium were determined from 5 mL of aliquot of the digestion mixture using an autoanalyser. Phosphorus was determined by adding ammonium molybdate/antimony potassium tartrate solution and ascorbic acid and the absorbance read at 880 nm. Calcium and magnesium were determined by adding 10 mL of 0.15% lanthanum chloride and analysis in an atomic absorption spectrophotometer.

Total carbon analysis

Total carbon was determined by oxidisation with concentrated sulfuric acid and 1 M aqueous potassium dichromate mixture with external heating, followed by titration against 0.2 M ferrous ammonium sulfate solution using 1,10 phenanthroline ferrous sulphate indicator (Anderson and Ingram 1993).

Table 2. Quality parameters of organic materials (grouping adapted from Palm et al. (2001)).

Sample name	Plant part	%N	%P	%K	%Ca	%Mg	%C	%PP	% lignin	% soluble carbon	C:N	Protein Binding capacity BSA mg/g plant material
Quality class I (High N, low lignin and PP*)												
Croton megalorapus	leaf	3.38	0.14	1.96	1.54	0.57	41.63	3.09	8.68	7.54	12.3	35.9
Senna spectabilis	leaflets	4.18	0.22	1.56	1.61	0.21	44.25	2.73	8.20	11.94	10.6	18.9
Ctotalaria ochroleuca	leaflets	5.32	0.24	1.57	0.91	0.44	45.46	3.13	3.55	10.44	8.6	22.1
Crotalaria grahamiana	leaflets	3.42	0.16	0.64	1.84	0.53	37.82	2.77	4.85	10.12	11.1	21.0
Gliricidia sepium	leaflets	3.79	0.16	0.90	1.89	0.81	43.67	2.87	10.77	13.80	11.5	29.2
Senna spectabilis	leaflets	3.42	0.17	1.27	1.88	0.18	46.45	3.68	9.64	12.85	13.6	11.5
Senna spectabilis	leaf	4.58	0.23	2.04	1.33	0.17	45.46	1.89	11.27	9.94	10.0	26.1
Sesbania sesban	leaf	4.48	0.24	1.13	5.34	0.49	37.02	2.30	2.54	15.10	8.3	30.2
Quality class II (High N, high PP, high lignin)												
Calliandra calothyrsus	leaflets	3.53	0.13	0.50	1.82	0.58	41.90	10.04	14.53	9.21	11.9	117.8
Calliandra calothyrsus	leaflets	3.20	0.10	0.49	1.23	0.39	44.48	9.46	15.79	9.46	13.9	197.8
Flemingia congesta	leaflets	2.90	0.18	0.46	1.64	0.41	40.41	8.63	16.11	11.35	14.0	171.3
Quality class II(High N, high PP, low lignin)												
Lantana camara	leaf	3.45	0.21	2.26	1.53	0.44	41.00	6.15	11.60	8.38	11.9	47.9
Calliandra calothyrsus	leaflets	4.09	0.16	0.60	1.38	0.55	44.45	9.54	8.84	7.60	10.9	163.8
Senna siamea	leaflets	2.93	0.13	0.53	2.39	0.13	44.85	7.23	11.27	9.78	15.3	24.1
Tithonia diversifolia	leaf	3.29	0.27	3.36	1.95	0.48	39.82	5.97	8.16	9.21	12.1	29.3
Calliandra calothyrsus	leaf	3.03	0.12	0.49	1.35	0.47	43.83	14.01	9.78	11.43	14.5	294.7
Calliandra calothyrsus	leaf	3.03	0.11	0.61	0.91	0.40	46.40	14.48	6.21	12.75	15.3	287.6
Calliandra calothyrsus	leaflets	3.10	0.10	0.49	0.89	0.35	46.28	14.77	12.09	12.04	14.9	322.0
Calliandra calothyrsus	leaf	2.61	0.08	0.48	0.99	0.41	45.08	12.26	12.93	10.42	17.3	280.4
Lantana camara	leaf	4.51	0.33	2.59	1.49	0.66	43.67	5.15	6.20	8.51	9.70	12.6
Tithonia diversifolia	leaf	4.25	0.26	4.03	1.93	0.41	37.68	4.85	4.56	9.53	8.9	24.1
Quality class II (High N, high lignin, low PP)												
Gliricidia sepium	leaflets	3.58	0.16	1.44	3.23	0.73	40.53	2.56	15.68	11.86	11.3	21.1
Cattle manure		2.54	0.62	3.56	1.08	0.68	36.98	1.05	17.27	3.74	14.6	48.3
Gliricidia sepium	leaflets	3.78	0.16	2.12	1.85	0.48	40.68	3.50	16.67	11.59	10.8	33.6
Quality class III (Low N, low lignin)												
Zea mays	stover	0.59	0.03	0.80	0.33	0.34	41.30	1.06	4.62	4.84	70.9	14.7
Senna siamea	leaflets	1.99	0.10	0.62	3.25	0.28	43.56	8.14	10.45	11.90	21.8	22.1
Saccharum officinarum	stover	1.22	0.15	2.18	0.28	0.10	40.16	1.51	4.72	4.85	33.4	18.9
Lantana camara	stems	0.95	0.07	1.33	0.32	0.11	42.57	1.48	16.40	3.11	45.0	21.0
Quality class IV (Low N, high lignin)												
Gliricidia sepium	stems	1.64	0.09	2.67	0.97	0.36	42.08	1.30	20.44	5.01	25.7	26.1
Sesbania sesban	stems	0.82	0.04	0.76	0.62	0.12	44.38	0.84	15.09	3.24	54.4	25.2
Eucalyptus saligna	leaf litter	1.03	0.03	0.43	0.95	0.13	46.13	10.83	23.68	8.94	44.8	182.9
saw dust		0.14	0.01	0.05	0.08	0.02	48.57	1.74	29.45	1.40	348.8	19.9

Water-soluble carbon analysis

Water-soluble carbon was obtained by wet oxidation using potassium dichromate. Twenty mL of deionised water was added to 0.03 g plant material in a glass bottle followed by hand shaking. The bottles were placed in a water bath at 100°C for 1 hour with occasional shaking. After filtration, 2 mL of 0.16 M potassium dichromate was added to 10 mL extract in digestion tubes. Another 10 mL of concentrated sulfuric acid was added while mixing on a vortex mixer. The digestion tubes were placed in a pre-heated block at 150°C for 30 minutes, then let cool. Samples were read on a spectrophotometer at 600 nm to obtain carbon concentration.

Total soluble polyphenols

Total soluble polyphenols were determined by the Folin-Ciocalteau method (Constantinides and Fownes 1994). This involved extraction of 0.1 g material with 50% methanol in a water bath at a temperature of 77–80°C for 1 hour. The extract was filtered into a 50 mL conical flask and made to volume with distilled water. Folin-Ciocalteau reagent (2.5 mL) and 10 mL of 17% sodium carbonate was added to 1 mL extract in a 50 mL conical flask, made to volume and left to stand for 30 minutes for colour development. Standard samples of known tannic acid concentration were treated in the same way, and absorbance of the standards and samples was read in a spectrophotometer at 760 nm. Concentration of samples was obtained by plotting absorbance against concentration of standard samples. Percent polyphenol (expressed as tannic acid equivalent) was calculated as:

$$\% \text{ total soluble polyphenols} = (C \times 250)/W$$

where C = corrected concentration of sample in mg mL^{-1}
W = moisture corrected weight of sample in g
250 = a dilution factor.

Lignin content analysis

Lignin was determined through acid detergent fibre (ADF) via Ankom Technology. Plant materials (0.5 g) were placed into fibre bags that were then sealed with a heat sealer. These were placed in an Ankom Machine into which was added 2 litres of extracting solution (solution of sulfuric acid and centyltrimethyl ammonium bromide). Extraction was done for 1 hour at 100°C. The samples were then washed with boiling water, followed by repeated washing with acetone to remove plant pigments. They were then oven dried at 80° C and weighed to determine ADF. The samples were further hydrolysed with 72% sulfuric for 3 hours and washed repeatedly with boiling water followed by drying at 80° C to obtain lignin plus ash. Ashing was done in a muffle furnace at 550°C for 3 hours to destroy lignin and obtain ash. Lignin was then obtained by weight loss upon ashing, using the following formula:

$$\% \text{ lignin} = (W2 - W3)/W1) \times 100$$

where $W1$ = moisture free weight of sample
$W2$ = lignin plus ash weight
$W3$ = ash weight.

Protein-binding capacity

Protein binding capacity of polyphenols was determined by extracting the material using 50% aqueous methanol at 95°C. The extract was centrifuged and applied to chromatographic paper, followed by reaction with bovine serum albumin (BSA). Bound BSA was stained with Ponceou S and its absorbance read at 525 nm followed by conversion of absorbance to protein units using a calibration curve (Handayanto et al. 1994).

Results and Discussion

The proximate analysis enabled the differentiation of the standard sample set into different quality classes depending on their nitrogen, lignin and polyphenol contents (Table 2). Materials in quality class II were further grouped into three categories depending on their polyphenol and lignin contents.

Nitrogen was linearly correlated with all parameters except K and PBC (Table 3). Thus, materials high in N (classes I and II) were also high in phosphorus, calcium and magnesium concentrations, but low in lignin concentration. Potassium concentration was, however, not correlated with N content. Class II materials (mainly *Calliandra calothyrsus*) were generally high in polyphenol contents, and had high protein-binding capacity. Class III materials were low in soluble carbon, polyphenols and protein-binding capacity (except for one material). Class IV materials comprised mainly stems and were generally low in nutrients but high in lignin.

Table 3. Simple linear correlation coefficients for quality parameters.

	%C	%N	%P	%K	%Ca	%Mg	% PP	% Lignin	% Soluble carbon	C:N	PBC
%C	1.00	–0.166	–0.518**	–0.554**	–0.419*	–0.455**	0.001	0.326	0.001	0.366*	0.353
%N		1.00	0.510**	0.275	0.500**	0.556**	0.683**	–0.539**	0.683**	–0.580**	–0.009
%P			1.00	0.687**	0.234	0.480**	0.073	–0.265	0.073	–0.352*	–0.250
%K				1.00	0.041	0.241	–0.173	–0.218	–0.173	–0.277	–0.464**
%Ca					1.00	0.371*	0.662**	–0.313	0.662**	–0.365*	–0.202
%Mg						1.00	0.364*	–0.267	0.364*	–0.443*	0.068
% PP							1.00	0.011	0.419*	–0.210	0.902**
% Lignin								1.00	–0.421*	0.561**	0.106
% Soluble carbon									1.00	–0.542**	0.278
C:N										1.00	–0.133
PCB											1.00

* and ** refer to significance at 5 and 1% confidence levels, respectively; PCB = protein-binding capacity of polyphenols; PP = total soluble polyphenols.

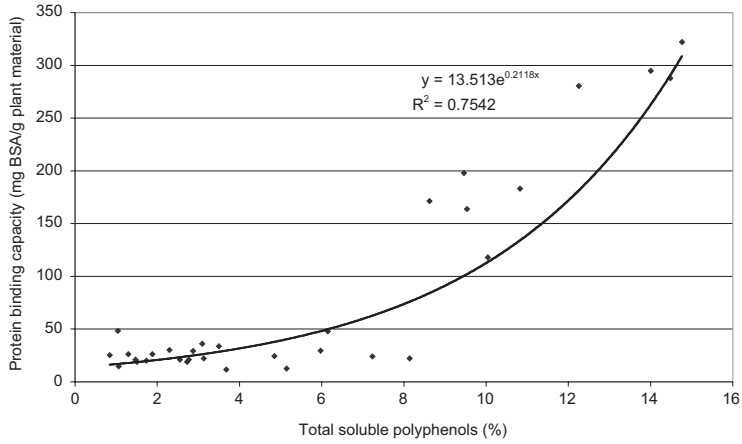

Figure 1. Relationship between total soluble polyphenols and protein binding capacity.

The relationship between protein-binding capacity and total soluble polyphenols is twofold (Fig. 1). From the data, it appears that the relationship can best be described using broken-stick models (see also Handayanto et al. (1997)), with an initial phase (up to about 8% total soluble PP) with few active polyphenols, followed by a linear relationship between total extractable PP and PBC. Polyphenol contents below ~8% resulted in protein-binding capacity < 50 mg BSA g^{-1}.

On the other hand, polyphenol contents above 10% resulted in PBC > 100 mg BSA g^{-1}. Correlation between soluble polyphenols and protein binding capacity ($r = 0.90$) was highly significant (Table 3).

Conclusion

Proximate analysis of the 32 organic materials showed that they covered the four resource quality classes that relate to nutrient release. Materials high in N were low in lignin and high in other nutrients (except K) and, as expected, the soluble polyphenols correlated significantly with protein-binding capacity at higher levels.

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