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RESEARCH ARTICLE

Mixing organic amendments with high and low C/N ratio influences nutrient availability and leaching in sandy soil

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Abstract:

Little is known about available N and P, microbial biomass and leachate N and P concentration in mixes of organic materials differing in C/N ratio. Sandy soil was amended with wheat straw (W, C/N 71) and cow manure (CM, C/N 7) either alone (100W and 100CM) or in different ratios (values are weight percentage of the organic materials): 75W-25CM, 50W-50CM, 25W-75CM. The control was unamended soil. Moist soil was incubated for 26 days, leaching was carried out on days 10 and 25. Soil was sampled on day 9 (before first leaching) and day 26 (after the second leaching). Cumulative respiration over 26 days was similar in the unamended control and 100CM, it increased with proportion of W in the amendments. Per kg of soil, amended soils did not differ in available N, microbial biomass N (MBN) and leachate inorganic N concentration, but available P and leachate inorganic P increased with proportion of CM in the amendment. However, available N, MBN and inorganic N in leachate per g N added was highest in 100W and lowest in 100CM. In contrast, available P and leachate P concentration per g P added increased with proportion of CM. Measured available N and leachate inorganic N were lower than expected values whereas measured available P and inorganic P in the leachate were higher than expected. In mixes, CM appears to reduce N mineralisation in wheat whereas W stimulates P release from cow manure.

Keywords: Available N; available P, leached N, leached P; cow manure, organic amendment mixtures, wheat straw.

1. Introduction

Organic amendments can be used as nutrient source for plants, but their effect on nutrient availability depends on properties such as C/N ratio and concentration of rapidly and slowly decomposable compounds (Palm and Sanchez, 1991; Berg and McClaugherty, 2004; Partey *et al.*, 2013; Zheng and Marschner, 2017). Mixing of organic amendments differing in chemical composition allows manipulating decomposition, mineralization and leaching. Incorporation of a high C/N ratio together with a low C/N ratio organic material can lead to lower rates of N mineralization, thereby reducing N leaching compared to low C/N ratio material alone (Vityakon *et al.*, 2000). Partey *et al.* (2013, 2014) showed that mixing maize (*Zea mays*) residues with high C/N ratio (37) with either *Tithonia diversifolia* (C/N ratio 14) or *Vicia faba* (C/N ratio 8) residues at a 1:1 ratio (w/w), tripled decomposition and N release rate compared to that of maize alone. Similarly, Singh *et al.* (2007) found that mixing Sesbania (C/N 16) with wheat straw (C/N 82) doubled decomposition rate and increased N availability and microbial biomass compared to wheat straw alone.

However, decomposition and nutrient release in mixes are not always predictable based on that of the individual materials (Handayanto *et al.*, 1997). Predicted values are calculated from mineralisation of individual species multiplied by their proportion in the mix. Interactions among species in mixes can lead to additive and non-additive responses, with non-additive responses being observed more often (Gartner and Cardon, 2004). In an additive response, measured mineralisation in the mix matches predicted values, whereas in a non-additive response, measured mineralisation in mixes is either lower or higher than expected values (Gartner and Cardon, 2004). Interactions between organic materials in mixes may depend on parameter studied. In mixes of four litter types, Bonanomi *et al.* (2010) showed that measured values of mass loss, N, Ca contents were lower than expected whereas measured values of K and Mg contents were higher. There are several factors driving the interaction among different organic materials in mixes, among which chemical composition is considered to be particularly important (Chapman *et al.*, 1988). For example, nutrients released from decomposable material can accelerate decomposition rate of less decomposable material in mixes in a synergistic response (Chapman *et al.*, 1988). On the other hand, inhibiting compounds such as phenolics and tannins in one material can retard decomposition of other materials (Dix, 1979). Further, chemical, physical and biological changes in mixes can influence the outcome of interactions in mixes (Gartner and Cardon, 2004).

Previous studies have focused on the effect of mixing of different organic materials on mass loss and nutrient contents (e.g., Xiang and Bauhus, 2007; Bonanomi *et al.*, 2010), but little is known about the effect of mixing of organic materials on microbial biomass, cumulative respiration and leachate nutrient concentration compared to expected values. The aim of this study was to determine the effect of organic amendment with different C:nutrient ratio (wheat straw with high C:nutrient ratio and cow manure low C:nutrient ratio) added singly or as mix on available N, P, microbial biomass N (MBN) and microbial biomass P (MBP) in a sandy soil and on inorganic N and P in leachate. Sandy soil was chosen to minimize sorption of nutrients to soil and thereby maximizing availability and leaching. The following hypotheses were tested: (i) due its low N and P concentration compared to cow manure, addition of wheat straw will result in lower N and P availability and N and P in leachate than with cow manure, (ii) in mixes, non-additive interactions will dominate, but depend on parameter assessed.

2. Materials and Methods

2.1. Sandy soil

A coarse sand from a sand pit in South Australia was used (Santos Ready Mixed Concrete Pty Ltd). The sand was air-dried at room temperature and sieved to particle size < 2 mm. The sand was incubated at 80% of water holding capacity (WHC) for 10 days at room temperature to activate soil microbes before mixing with organic materials. Butterly *et al.* (2010) showed that 10 days after rewetting air-dry soil, soil respiration is stable. For soil analyses, see section 2.4.

2.2. Organic materials

Two types of organic materials were used: mature wheat straw (*Triticum aestivum* L.) and cow manure (Table 1), referred to as W and CM. The organic materials were oven-dried at 40 °C, ground and sieved to particle size from 0.25 to 2 mm. For analyses of properties, see section 2.4.

2.3. Experimental design

The amendments were used alone (100W and 100CM) or mixed at different ratios (percentage in mix): 75W-25CM, 50W-50CM, 25W-75CM. Then, the organic materials were thoroughly mixed into the sandy soil at the rate 10 g dry weight kg^{-1} dry soil. Total N, P and C in each treatment were calculated based on total N, P and C of W and CM and their proportion in each treatment. The control was unamended sand. The sand-organic material mixture (30 g) was placed in PVC cores (1.85 cm radius, 5 cm height) and incubated at 80% WHC in the dark at 22-25 °C. A preliminary experiment had shown that soil respiration was maximal at this water content in this soil. Leaching was carried out on days 10 and 25 with four replicates per treatment and leaching event. Cores were destructively sampled on day 9 (before the first leaching) and day 26 (after the second leaching) for determination of available N, P and MBN, MBP. The cores to be sampled on day 9 were placed in 1L glass jars for respiration measurement until day 9. The other set of cores was incubated on trays in the same environmental conditions as the cores in the jars. The cores on the trays were leached on day 10, dried as described below and placed into the glass jars for respiration measurement until the second leaching on day 25. The following day (day 26), the soil in the cores was destructively sampled. Leachate was analyzed for inorganic N, inorganic P and water extractable organic carbon. The water content was maintained at 80% WHC throughout incubation by adding reverse osmosis (RO) water by weight.

For leaching, 50 ml RO water was added in five 10 ml aliquots. Between additions, the water was allowed to drain from the soil surface before the next aliquot was added. This amount of RO water was used to obtain sufficient leachate for the analyses (42–45 ml). After the first leaching, the cores were placed in an oven at 40 °C for about 4 h until the water content was 80% WHC. The expected value for the measured parameters in the organic material mixes was calculated based on average concentration of separate organic amendment according to the following equation (Gartner and Cardon, 2004):

Expected value = (Proportion of W in mix * concentration in sole W) + (Proportion of CM in mix*concentration in sole CM). Equation 1

2.4. Analyses

Soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil pH and electrical conductivity were determined in a 1:5 soil: water extract after 1 hour end-over-end shaking at 25 °C (Rayment and Higginson, 1992). WHC of the sand alone or amended with the organic materials was measured using a sintered glass funnel connected to 100 cm water column (matric potential -10 kpa) as described by Wilke (Wilke, 2005). With organic materials, WHC was about three-fold higher than sandy soil alone. Total organic carbon in soil and residues was determined using the dichromate oxidation and titration method (Walkley and Black, 1934). For measurement of total N, residues were digested in H_2SO_4, then total N in the digest was measured by a modified Kjeldahl method (Vanlauwe *et al.*, 1996). For total P, residues were digested in HNO_3 and $HClO_4$ (6:1). Total P in the digest was measured by the phosphovanado-molybdate method (Hanson, 1950).

Due to the low respiration rate of the sand-organic material mixes and the detection limit of the gas analyser (2% CO_2), the CO_2 concentration in the headspace of the jars was measured every two days using a Servomex 1450 infrared gas analyser (Servomex, UK) as described in Setia *et al.* (2011). After each CO_2 measurement (t1), the jar was opened to release the air from the headspace using a fan followed by determination of the CO_2 concentration (t0). The CO_2 respired every interval was calculated as the difference in CO_2 concentration between t1 and t0.

Available N was measured after 1 h end-over-end shaking with 2 M KCl at a 1:5 soil extractant ratio. The nitrate-N concentration in the extract was measured after Miranda *et al.* (2001), the ammonium concentration after Willis *et al.* (1996). Available N was measured colorimetrically at 450 and 685 nm for nitrate and ammonium.

Available P was extracted by the anion exchange resin method (Kouno *et al.*, 1995), and P concentration was determined colorimetrically at 712 nm (Murphy and Riley, 1962).

MBN was extracted by the fumigation extraction method (Vance *et al.*, 1987). Soil samples were fumigated with chloroform for 24h followed by shaking with 0.5 mol L^{-1} K_2SO_4 at 1:4 soil extractant ratio for 1h. For MBN determination, ammonium-N was determined in the extract (Moore *et al.*, 2000). MBN was calculated as the difference in ammonium-N concentration between fumigated and non-fumigated samples divided by 0.57 as suggested by Moore *et al.* (2000).

MBP was determined by the anion exchange method of Kouno *et al.* (1995) with the modification that hexanol was used for fumigation instead of chloroform. P concentration was determined colorimetrically as described above for available P (Murphy and Riley, 1962). MBP was calculated by subtracting the P content of fumigated from un-fumigated samples.

Inorganic N, P in the leachate were determined using the same analytical methods as for available N, available P. Organic C in the leachate was determined by oxidising with $K_2Cr_2O_7$ and H_2SO_4. The remaining $K_2Cr_2O_7$ was titrated with acidified $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Anderson and Ingram, 1993).

Cumulative respiration, available N, P, MBP and MBN, leached inorganic N and P and organic C were expressed per g or kg of soil or per g C, N and P added. Expression per g nutrient added can be used to assess its availability/decomposability which is particularly useful in mixes of organic materials.

2.5. Statistical analysis

The data of available N, P, MBN and MBP in soil and inorganic N, P and WEOC in leachate was analysed by one-way repeated measures Analysis of Variance (ANOVA). Cumulative respiration was analysed by one-way ANOVA. Mean values of four replicates at a given sampling time were compared using Tukey's multiple comparison tests, significance refers to $P \leq 0.05$. Properties of organic materials were compared by t test. Statistical analysis was carried out in IBM SPSS Statistics 24.

3. Results

Sandy soil alone had the following properties: 98% sand, 1% silt, 1% clay, $EC_{1:5}$ 14.3 µS cm^{-1}, $pH_{1:5}$ 6.3, WHC 0.008 g water g^{-1} soil, TOC 0.18 g kg^{-1}, available N 11.93 mg kg^{-1} and available P 0.36 mg kg^{-1}. In W, total organic N was almost three times higher than in CM (Table 1). But total N and P in CM were more than three times higher than in W. Therefore, total N and P added increased with increasing proportion of CM in the mix, whereas total organic C added decreased (Table 2).

Table 1. Selected properties of organic materials used (n=5, mean ± standard error). Within column, means followed by different letters are significantly different ($P \leq 0.05$).

Organic materials	Total C	Total N	Total P	C/N	C/P
	g kg^{-1}				
Cow manure	136.1 a ± 9.4	19.7 a ± 0.9	5.6 a ± 0.1	7 a	24 a
Wheat straw	391.2 b ± 24.5	5.5 b ± 0.4	1.7 b ± 0.1	71 b	228 b

Table 2. Total C, N and P added in treatments.

Treatment	Total C (g C kg^{-1} mix)	Total N (g N kg^{-1} mix)	Total P (g P kg^{-1} mix)
100W	3.91	0.05	0.02
75W-25CM	3.27	0.09	0.03
50W-50CM	2.64	0.13	0.04
25W-75CM	2.00	0.16	0.05
100CM	1.36	0.20	0.06

3.1 Cumulative respiration

Compared to the unamended control, cumulative respiration per g soil was highest in 100W where it was 12 fold higher than the control (Figure 1). Cumulative respiration in 100CM was similar as in the control. Cumulative respiration per g C added was higher in mixes with 50% or more W than in mixes with lower proportion of W (Figure 1).

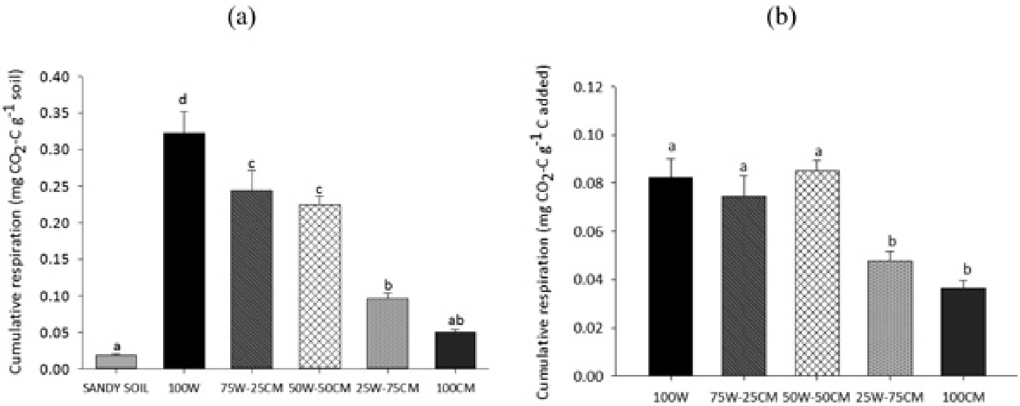


Figure 1. Cumulative respiration after 25 days in mg CO_2-C g^{-1} soil (a) and mg CO_2-C g^{-1} C added (b) in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. Bars with different letters are significantly different (n=5, $P \leq 0.05$).

3.2. Available N and P and microbial biomass before the 1st and after 2nd leaching

In amended soils, available N and P per kg soil were higher before the 1st leaching than after the 2nd leaching (Table 3). On day 9, available N in amended soils was higher than in the un-amended control except for 50W-50CM and 100CM, however there was no significant difference among treatments on day 26.

Table 3. Available N, available P and microbial biomass N and P (mg kg^{-1} soil) in soil before 1st (day 9) and after the 2nd leaching event (day 26) in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$)**.**

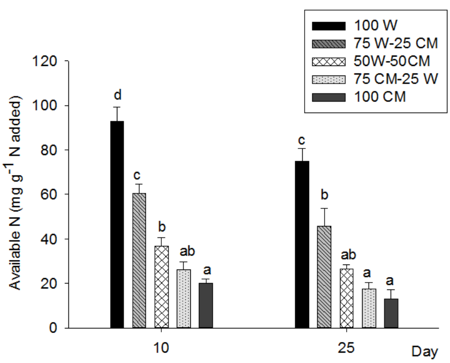
Treatment	Available N		Available P		MBN		MBP	
	mg kg^{-1} soil							
	Day 9	Day 26	Day 9	Day 26	Day 9	Day 26	Day 9	Day 26
Control	2.94 a	2.93 a	0.13 a	0.40 a	0.09 a	0.23 a	0.03 a	0.05 a
100W	5.10 b	3.94 a	0.77 a	0.56 a	5.47 b	0.78 a	1.61 ab	0.11 a
75W-25CM	5.47 b	3.75 a	3.71 b	2.32 b	2.41 a	0.03 a	2.85 ab	1.40 ab
50W-50CM	4.63 ab	3.29 a	8.11 c	5.21 c	2.39 a	0.64 a	2.06 ab	0.46 a
25W-75CM	5.47 b	2.52 a	11.05 d	6.87 d	0.81 a	0.02 a	2.75 ab	0.57 a
100CM	3.93 ab	2.15 a	16.54 e	7.62 d	0.72 a	0.44 a	3.55 b	3.45 b

Available P per kg soil on days 9 and 26 in amended soils was higher than that the control except for 100W. It increased with proportion of CM in mixes (Table 3). Available P was highest in 100CM on day 9 but similar in 25W-75CM and 100CM on day 26.

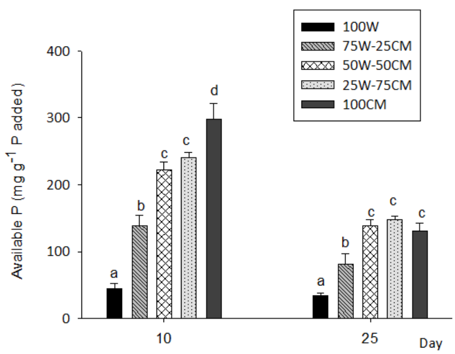
MBN per kg soil on day 9 was highest in 100W where it was 50 fold higher than in the control, but there was no significant difference among treatments on day 26 (Table 3). MBP on days 9 and 26 was higher than the control only in 100CM.

Available N per g N added reflected an opposite pattern than available P per g P added (Figure 2). Available N per g N added was highest in 100W where it was about five-fold higher than in 100CM (Figure 2). It decreased with proportion of W in the mix until 50W-50CM. There was little difference in available N between treatments with 50% or more percent CM. Differences among treatments were greater on day 9 than day 26.

(a)



(b)



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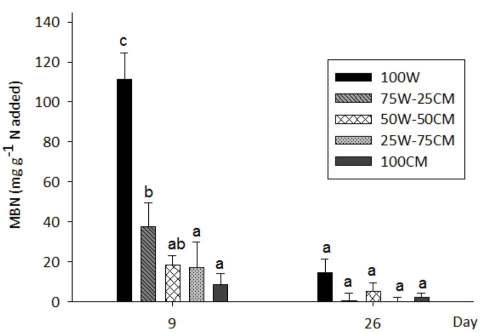


Figure 2. Available N per g N added (a), available P per g P added (b), MBN per g N added (c), before 1st leaching (day 9) and after the 2nd leaching (day 26) in sandy soil amended with wheat straw or cow manure only and both at different ratios. At a given sampling time, bars with different letters are significantly different (n=5, $P \leq 0.05$).

Available P per g P added on day 9 was highest in 100CM where it was more than six-fold higher than in 100W, followed by 25W-75CM and 50W-50CM (Figure 2). Available P per g P added in 75W-25CM was only a half of that in 100CM. On day 26 after the 2nd leaching, available P per g P added was lower in 75W-25CM and 100W than in 100CM, however there was no significant difference in mixes with ≥ 50% CM.

MBN per g N added on day 9 was highest in 100W where it was 12 times higher than in 100CM followed by 75W-25CM where it was about a third of that in 100W (Figure 2). MBN per g N added did not differ among treatments with ≤ 50% W. On day 26, MBN per g N added was very low and did not differ among treatments (Figure 2).

MBP per g P added in did not differ among treatments for both leaching events (Table 4).

Table 4. MBP per g P added before 1st (day 9) and after the 2nd leaching event (day 26) in sandy soil amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$)**.**

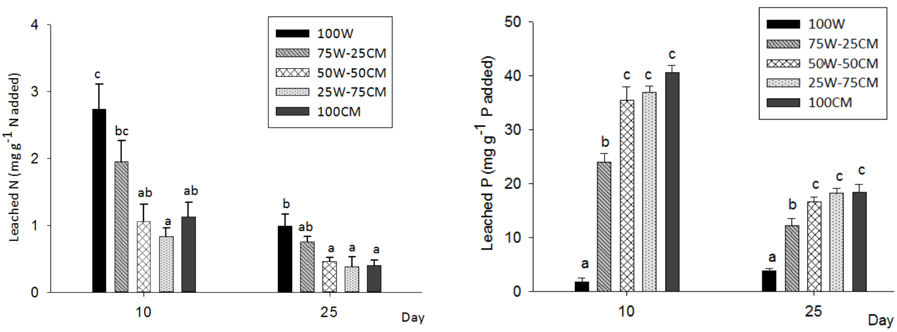
Treatment	**MBP**	
	mg g^{-1} P added	
	Day 10	Day 25
100W	93.9 a	6.4 a
75W-25CM	106.7 a	52.3 a
50W-50CM	56.6 a	12.7 a
25W-75CM	59.8 a	12.4 a
100CM	63.8 a	61.97 a

3.3 Inorganic N and P in leachate of both leaching events

Leachate nutrient concentration was lower in the 2nd leaching compared to the 1st (Table 5, Figure 3). For both leaching events, leached inorganic N per kg soil was low and did not differ among treatments (Table 4). Leached inorganic P on days 10 and 25 was similar in the control and 100W, where it was up to 40-fold lower than in the other treatments (Table 4). In amended treatments with ≤75% W, inorganic P in leachate increased with proportion of CM and was about twice as high on day 10 than day 25. WEOC in leachate per kg soil on day 10 was lowest in the control and highest in 100W (Table 4). It decreased with proportion of W up to 50W-50CM, but was similar in treatments with ≥ 50% CM. WEOC per kg soil decreased up to four-fold in amended soils from day 10 to day 25. On day 25, WEOC was higher than the control only in 100W and 75W-25CM.

Table 5. Inorganic N and P and water-extractable organic C (WEOC) of leachate (mg kg^{-1} soil) of the 1st and 2nd leaching in sandy soil alone or amended with wheat straw or cow manure only and both at different ratios. On a given day, values with different letters are significantly different (n=5, $P \leq 0.05$).

Treatment	Inorganic N		Inorganic P		WEOC	
	mg kg^{-1} soil					
	Day 10	Day 25	Day 10	Day 25	Day 10	Day 25
Control	0.15 a	0.07 a	0.06 a	0.04 a	10.89 a	9.20 a
100W	0.17 a	0.05 a	0.03 a	0.07 a	90.65 d	17.84 c
75W-25CM	0.18 a	0.07 a	0.64 b	0.33 b	73.94 c	14.03 b
50W-50CM	0.13 a	0.06 a	1.29 c	0.60 c	56.23 b	12.35 ab
25W-75CM	0.13 a	0.06 a	1.70 d	0.84 d	50.82 b	11.79 ab
100CM	0.22 a	0.08 a	2.26 e	1.02 e	46.40 b	11.82 ab



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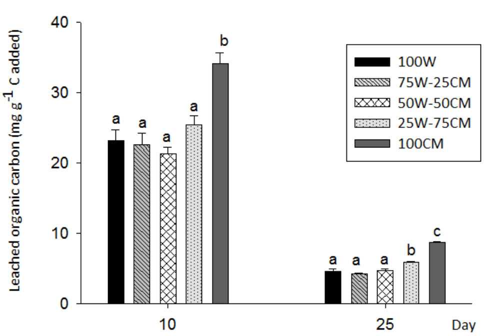

Figure 3. Leached inorganic N per g N added (a), inorganic P per g P added (b) and organic C per g C added (c) in the 1st (day 10) and the 2nd leaching event (day 25) in sandy soil amended with wheat straw or cow manure only and both at different ratios. At a given sampling time, columns with different letters are significantly different (n=5, $P \leq 0.05$).

Leached N per g N added was higher in 100W than in treatments with ≤ 50% W (Figure 3). It was about two to three-fold higher in the first than the second leaching event. Compared to 100W, leached P in 100CM was 12-fold higher on day 10 and five-fold higher on day 25. In both leaching events, leached P per g P added was higher in amendments with ≥50 % CM than those with lower proportion of CM (Figure 3).

On days 10 and 25, leached organic carbon per g C added was highest with 100CM where it was ≥ 30% higher than in the other treatments (Figure 3). Leached organic C per g C added was about four-fold lower on day 25 than day 9.

3.4 Measured compared to expected values

For both leaching events, measured available N, leached N and MBN per g N added and leached organic C per g C added were lower than expected values (Figure 4). Measured values were 20-40% lower for available N, 40-98% for MBN, 10-50% and 20-30% for leached N and organic C, respectively.

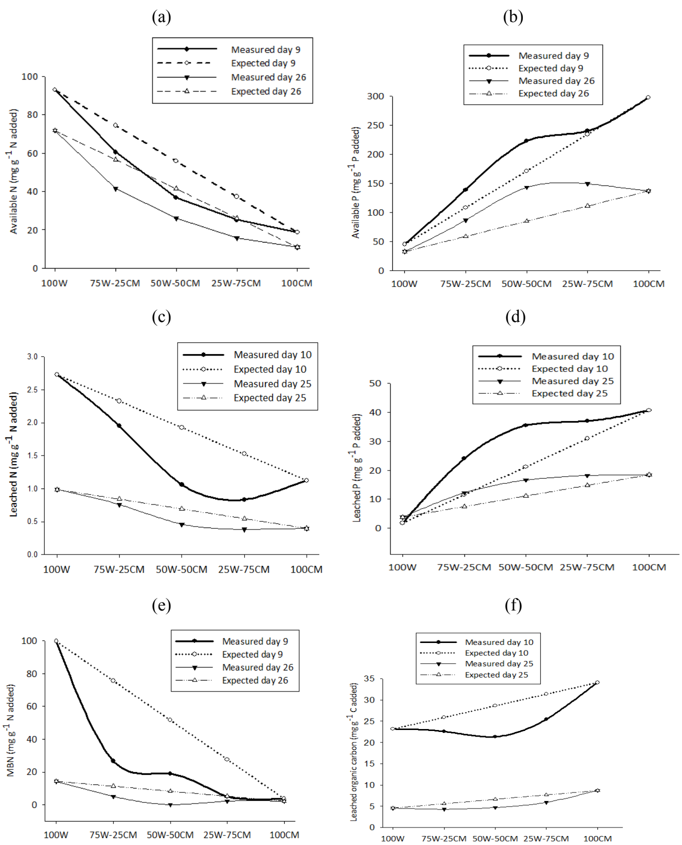

Figure 4. Expected and measured available N (a), available P (b) before 1st leaching (day 9) and after the 2nd leaching (day 26), and leached inorganic N (c), leached inorganic P (d), MBN (e), leached organic C (f) in the 1st (day 10) and the 2nd leaching event (day 25) in sandy soil amended with wheat straw or cow manure only and both at different ratios expressed in mg per g C, N and P added.

In contrast, measured available P and leached P per g P added were higher than the expected values for both leaching events (Figure 4). Measured available P and measured leached P were 2-70% and 20-100% higher than expected values, respectively.

3. Discussion

Based on this study, the first hypothesis (due its low N and P concentration compared to cow manure, addition of wheat straw will result in lower N and P availability and N and P in leachate than with cow manure) has to be declined, but the second hypothesis (in mixes, non-additive interactions will dominate, but depend on parameter assessed) can be confirmed..

3.1. Comparison 100W and 100CM

Wheat straw is generally considered to be slowly decomposable because of its high C/N and C/P ratio and high proportion of structural carbohydrates (Alexander, 1977; Xiao *et al.*, 2001). But in this study, CM was less decomposable than W despite its high N and P content. Manure is the result of microbial decomposition in the gut of animals and may be further decomposed during storage. Thus, it contains very little rapidly decomposable compounds and has a low C/N and C/P ratio. The highly decomposed state of the manure explains why addition of CM did not increase cumulative respiration compared to the unamended control. This also explains the low organic C concentration per kg soil in the leachate in 100CM compared to 100W.
CM contained three-fold more total N and P than W, but when expressed per kg of soil, available N and leached inorganic N was similar in 100CM and 100W and MBN on day 9 was lower in 100CM than 100W. On the other hand, available P and leached P were 10 and 15-fold higher in 100CM than 100W. Further, MBP on day 26 was 26-times higher in 100CM than 100W. These differences in nutrient release between the two amendments were also evident when available and leached N and P are expressed per g N and P added. The low N availability and leached N in 100 CM suggests that N in CM is in slowly decomposable compounds such as lignin. In contrast, a large proportion of P in CM appears to be in water-soluble and rapidly decomposable forms. Manures have been shown to contain large amounts of P when animals are fed a P-rich diet (Morse *et al.*, 1992). Per g C added, leached organic C was higher in 100 CM than 100W, whereas cumulative respiration was lower. This suggests that compounds in leached organic C of 100CM were slowly decomposable, e.g. aromatic compounds.

3.2 Mixes

To better understand nutrient dynamics in mixes, the following discussion is about available and leached N and P and MBN, MBP per g N and P added. As expected from the data of 100W and 100CM, available N, MBN and leached N decreased with proportion of wheat straw whereas available and leached P increased. However, the decrease or increase were not linear as the comparison between measured and expected values shows. The lower than expected available and leached N and MBN suggests that presence of CM inhibits mineralisation of organic N in wheat straw, possibly through aromatic compounds that either inhibit microbes or form stable compounds with proteins (Lucchini *et al.*, 1990). The higher than expected available and leached P indicates that presence of W enhanced release of P from CM. Increased release could be due to changed physical environment (e.g. better accessibility of CM particles). Another possible reason is that microbes decomposing W stimulated organic P mineralisation in CM to satisfy their P demand.

4. Conclusions

This study showed that addition of cow manure to soil may not result in high N leaching despite its low C/N ratio. The study further showed that mixing of cow manure and wheat straw can be used to reduce N leaching but may enhance P leaching. The high P availability and P leaching potential of cow manure could be beneficial for crops in P deficient soils, but may also have negative effects by increasing eutrophication. We used sand to minimize sorption of nutrients to soil particles. In soils with higher silt and clay content, N and P leaching is likely to be lower than in this study because released N and P are bound to clay minerals as well as organic matter.

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