

0.1 Preliminary incubation

The preliminary incubation was designed to examine the possible effect of two LTTs, ORG and MIN, on SOM properties during a one week incubation, using two different amendments with differing lability.

0.1.1 Dynamics of SOM properties in non-amended samples

Microbial biomass and respiration

both LTTs had seen high Resp rates (Fig 1) in the first 24 h of incubation, with peak rates during the first hours of incubation, quickly decreasing to reach steady values of less than 20 $mg\ CO_2-C * kg^{-1} * day^{-1}$ from 48 h onward. A pulse of Resp was observed in Min, peaking at 74 $mg\ CO_2-C * kg^{-1} * day^{-1}$ after 7 h and a significantly smaller peak of 57 $mg\ CO_2-C * kg^{-1} * day^{-1}$ was concurrently recorded for Org. interestingly, Resp rates were lower in Org during the first 96h of incubation. It is possible that peak Resp for Org had occurred at some point between the 2h and 7h sampling, thus being undetected. Our main experiment indeed showed peak Resp after 4h in control samples (results in following chapter). Still there is little reason to believe that an earlier peak in Resp would have been exclusively observed in Org as these two LTTs presented otherwise similar patterns. following from Resp rate data, cumulative respiration (Fig. 2) was significantly higher in Min throughout most of the incubation, with a 23% average increase over Org cumulative Resp values throughout the incubation.

A slight and evidently statistically insignificant, increase in MBC (Fig 3) was observed in Min samples after 24 h of incubation, followed by slight decrease in the next 3 days. In sharp contrast, in Org samples, MBC had seen a substantial increase of 100 $mg * kg\ soil^{-1}$ between 24-48h of incubation. This increase marked a peak of MBC in Org, after which MBC levels declined sharply to reach a value of 370 $mg * kg\ soil^{-1}$, same as in Min.

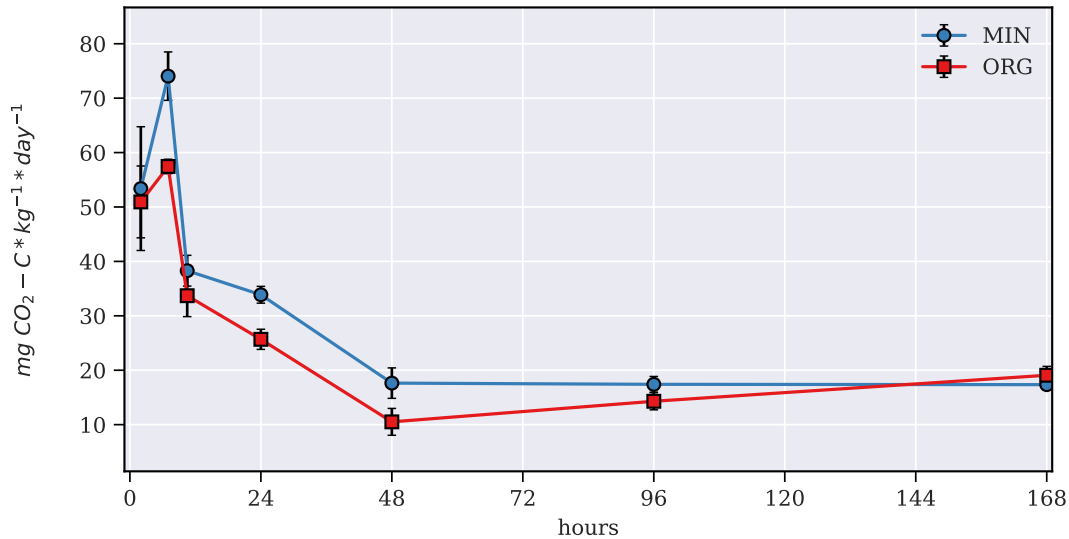


Figure 1: CO_2 respiration in non-amended samples

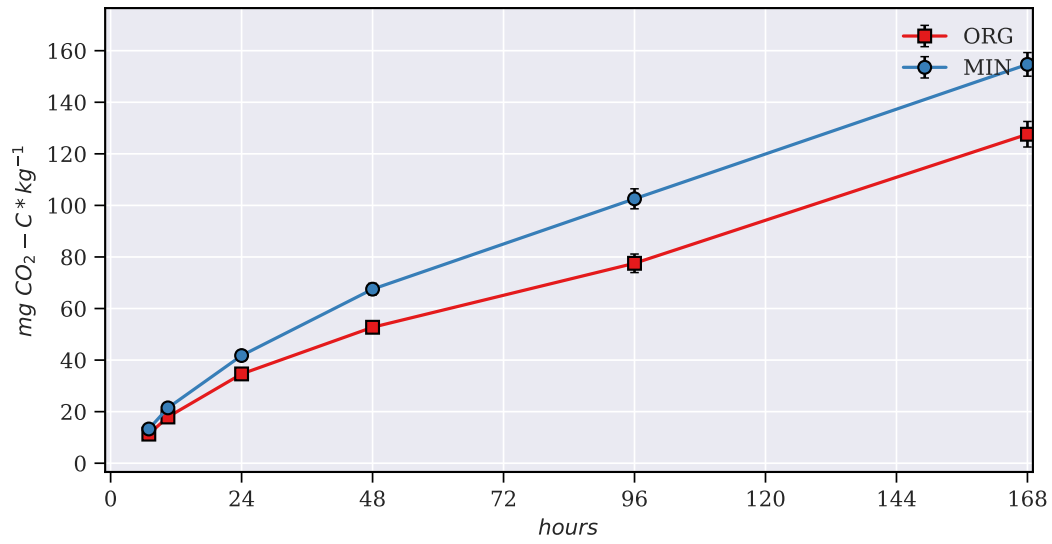


Figure 2: cumulative CO_2 respiration in non-amended samples

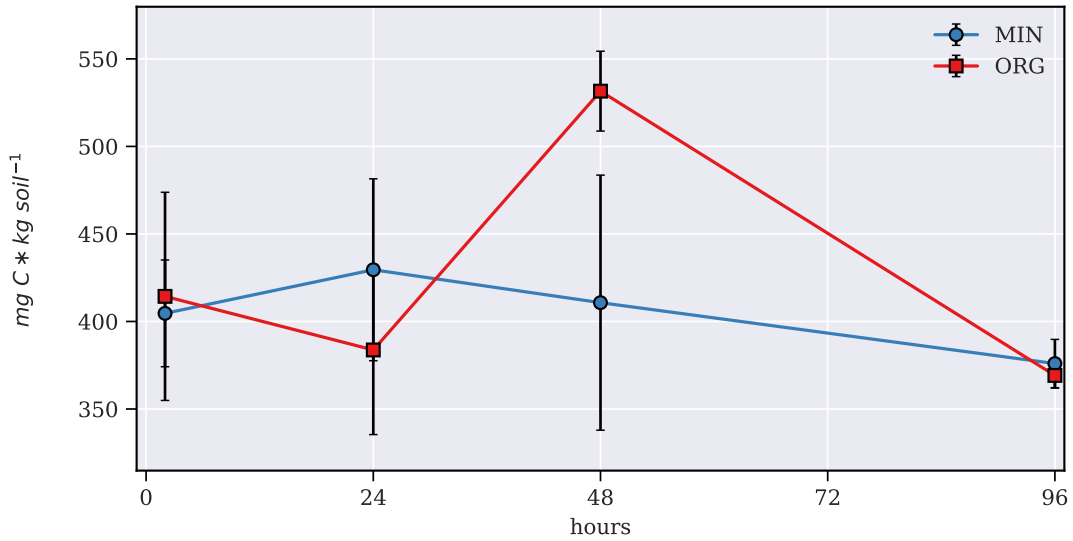


Figure 3: MBC in non-amended samples

WEOC

WEOC (Fig 4) was higher in Org throughout the incubation, with the exception of 48h. Initial levels of WEOC were more than 50% higher in ORG, with this percentage decreasing to 30% after 96 h of incubation. In both LTTs, WEOC was almost without change in the first 24 h, subsequently dropping sharply to reach a relatively similar value of $20\ mg * kg\ soil^{-1}$ in both LTTs after 48 h of incubation. This sharp decrease was followed by a substantial increase in the next 48 h, finally reaching values slightly, yet significantly higher than initial values. It is worth noting the opposite trends between MBC and WEOC, particularly in Org, whereby a sharp decrease in WEOC between 24-48 h was accompanied by a similarly sharp increase in MBC, and the same (inverse) contrast was observed in the following 48 h.

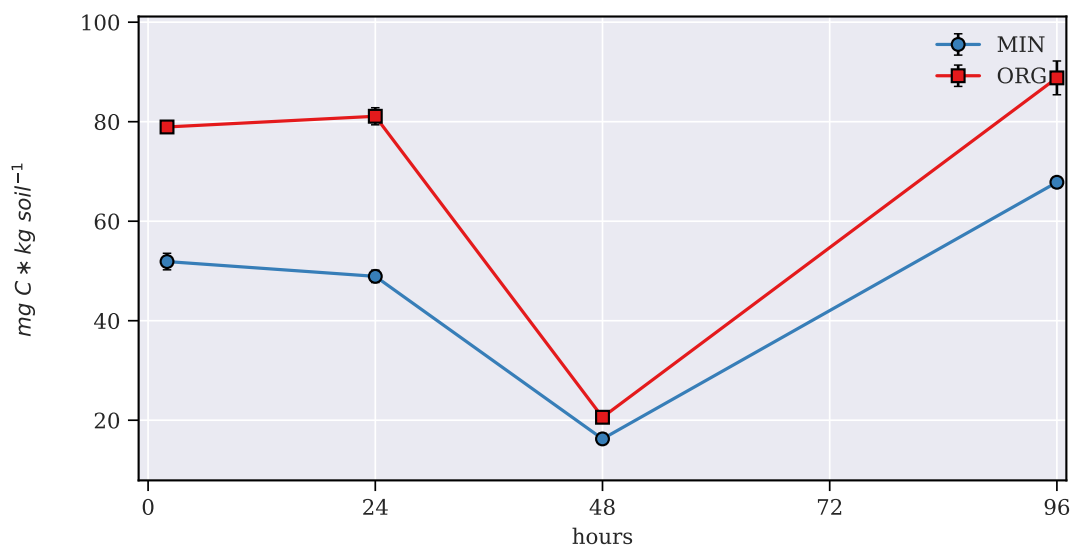


Figure 4: WEOC in non-amended samples

HWE total Carbon and Carbohydrate-C

Org had sustained significantly higher levels of HWE-C (Fig 5) and HWES-C (Fig 6) compared with Min throughout the entire incubation, with Org values 50 and 30% higher than Min, for HWE-C and HWES-C respectively. HWE-C and HWES-C had followed very similar patterns throughout the incubation, in both LTTs, while the dynamics of HWE-C fluctuated more sharply in the first 96h in Org but not so much in Min which presented strong concurrence in time dependent changes between HWE-C and HWES-C (pearson $r = 0.97$ for first differences in Min, compared with 0.91 for Org). These concurrent dynamics suggest that HWES-C comprised a relatively constant fraction of HWE-C in control samples of these two LTT, throughout the incubation period. This fraction, calculated by averaging the ratio of HWES-C-to-HWE-C across all sampling events, yielded a mean of 0.33 and 0.29 in Min and Org respectively, with a ± 0.01 associated error.

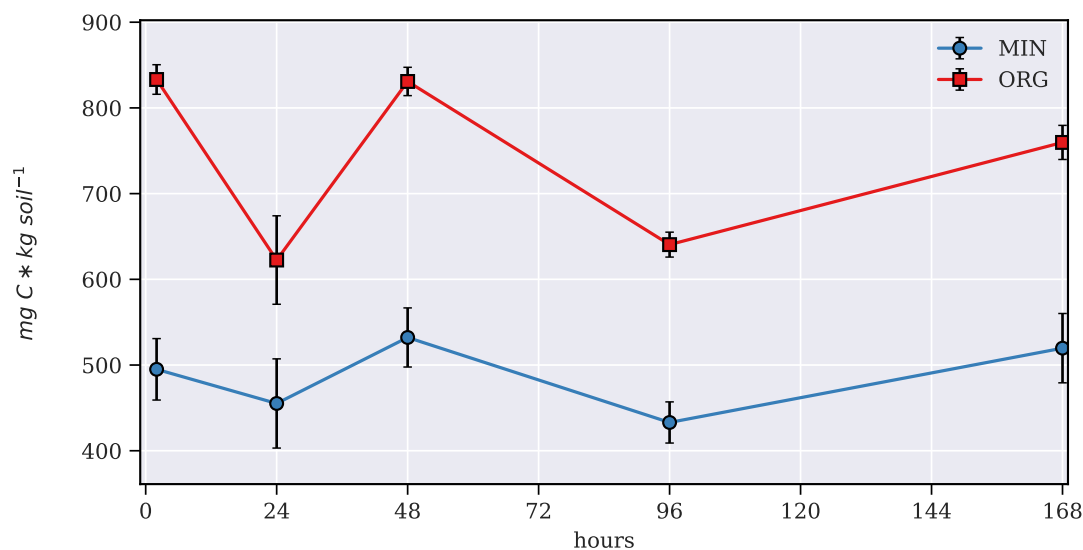


Figure 5: HWE-C in non-amended samples

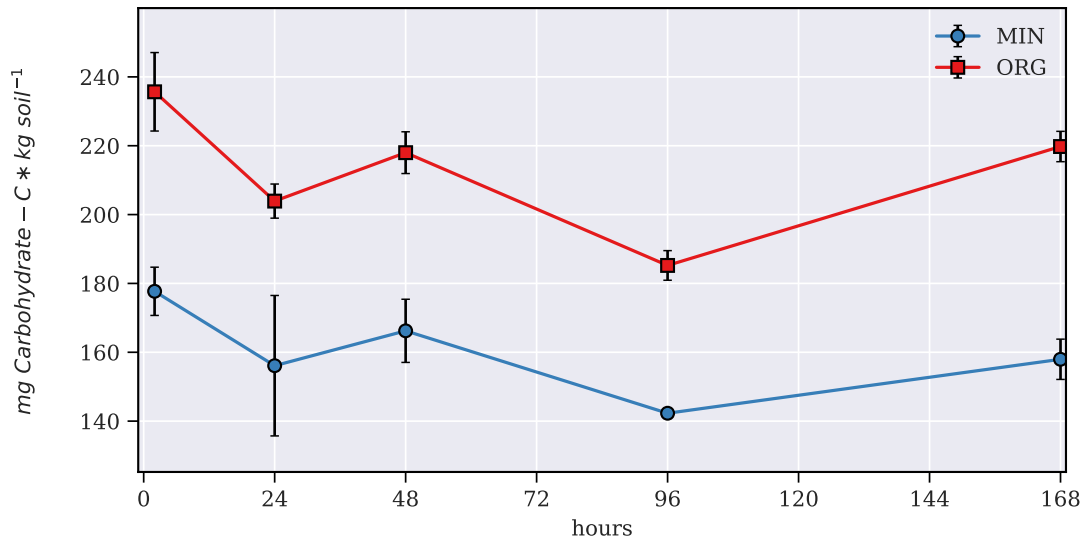


Figure 6: HWES-C in non-amended samples

Ergosterol

Min presented a slight, insignificant decrease in Erg during the incubation period, with a mean Erg concentration of $9 \text{ mg} * \text{kg soil}^{-1}$, while Org sustained a considerable reduction in Erg concentrations from $13 \text{ mg} * \text{kg soil}^{-1}$ in the first 24 h to $10.5 \text{ mg} * \text{kg soil}^{-1}$ by the end of the incubation (Fig. 7). the ratio of Erg-to-MBC saw some fluctuations in Org during the incubation, yet these were all statistically insignificant. this ratio remained practically steady in MIN samples (Fig 8). the average Erg-to-MBC ratio in ORG and MIN samples was 3 and 2.36% respectively.

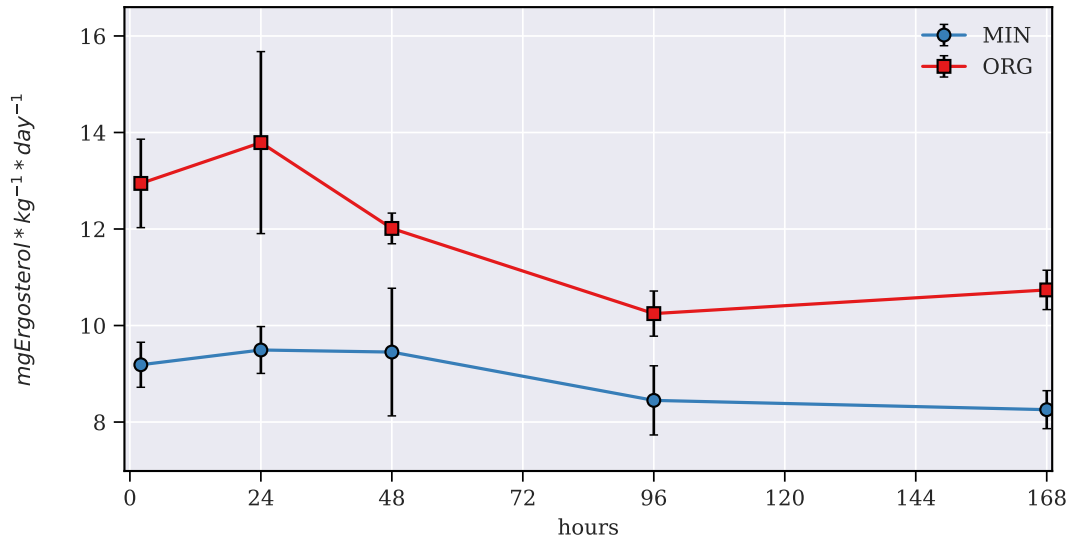


Figure 7: Ergosterol in non-amended samples

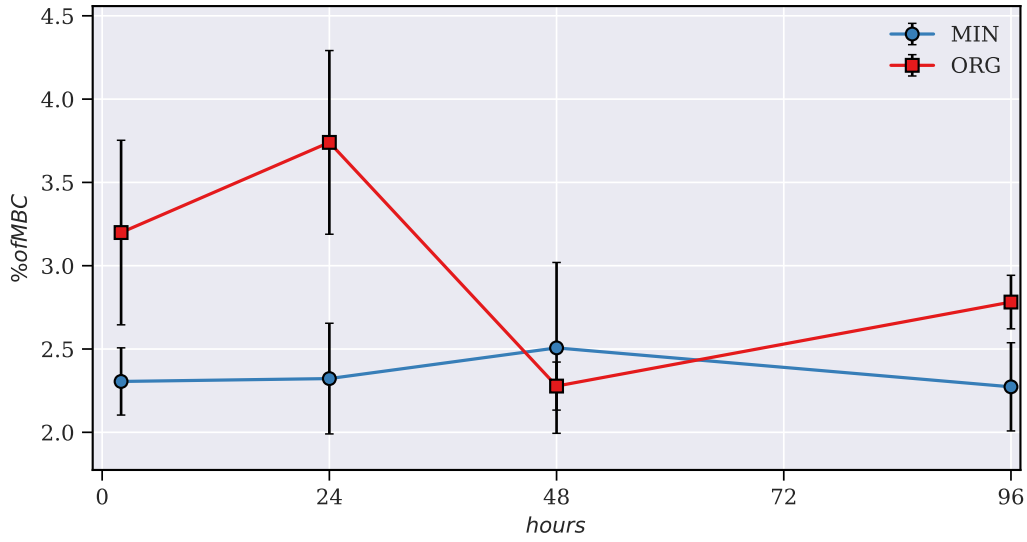


Figure 8: Ergosterol-to-MBC in non-amended samples

0.1.2 Dynamics of SOM properties in Straw and KW Compost amended samples

MB Carbon and Respiration

Org had seen an initial increase of 200 and 400 $mg * kg\ soil^{-1}$ MBC over control samples, following the addition of both Str and KWC respectively (Fig 10) , while for Min a smaller increase was observed after Str application. Data is missing for Min on the first sampling of KWC amended samples. Nonetheless, the otherwise parallel MBC trends between the two LTTs (in KWC amended samples) throughout the rest of the incubation (Fig. 9) , suggest a substantial initial increase of 400 $mg * kg\ soil^{-1}$ over control samples (absolute value of 600 $mg * kg\ soil^{-1}$ MBC at the first sampling) in Min+STR samples. In KWC amended samples, the initial MBC increase (assuming a value of 400 $mg * kg\ soil^{-1}$ Cn_MBC for Min samples) was followed by a general decrease in both LTTs, suggesting the favorable effect of KWC on MBC, was short lived, at least in the short-term period recorded in this incubation. Org+KWC had seen a decrease in Cn_MBC from the initial 382 to 250 $mg * kg\ soil^{-1}$ after 96h of incubation, while Min saw a similar or greater decrease across 4 days of incubation finally reaching a Cn_MBC value of 110 $mg * kg\ soil^{-1}$. Moreover, 24 h after the start of the incubation, MBC in Min+KWC samples had decreased by almost 100 $mg * kg\ soil^{-1}$ below the level of corresponding control samples, suggesting a short-term negative effect of KWC on MBC. In contrast with KWC, STR amendment had initially caused relatively small and little-to-no increases over control samples in Org and Min respectively, while seeing a sharp increase of more than 600 $mg * kg\ soil^{-1}$ in both LTTs after 24 h (50% higher than initial increase in KWC amended samples).

Resp dynamics in the two STTs seem to concur with their respective MBC dynamics (Fig 11). KWC Resp rates peaked immediately at the onset of the incubation and subsequently decreased rapidly, reaching control normalized values of close to 20 $mg\ CO_2-C * kg^{-1} * day^{-1}$ after 48 hours in both LTTs (Fig 12 B), while STR Resp rates peaked 7 and 10 h after incubation began, in Min and Org respectively (Fig 12 A). Indeed Resp data in KWC is missing for the 7h (as well as 24 h) sampling event, so it is possible that peak rates would have been detected after 7h of incubation. Nonetheless, the substantial difference of more than 150 $mg * kg\ soil^{-1}$ (75%) between the 2h and 10h sampling suggest that, even if this was true, this peak would probably not have been much higher than the observed peak. This intense initial respiration

response followed by rapid decline towards control respiration rates, corresponds to an initial MBC increase in these KWC amended samples. Similarly, a somewhat delayed Resp peak in STR amended samples and relatively high Resp rates sustained throughout the larger part of the incubation, concur with substantial growth in the first 24 h of incubation as well as with the high levels of MBC sustained through the next 72 h in these samples.

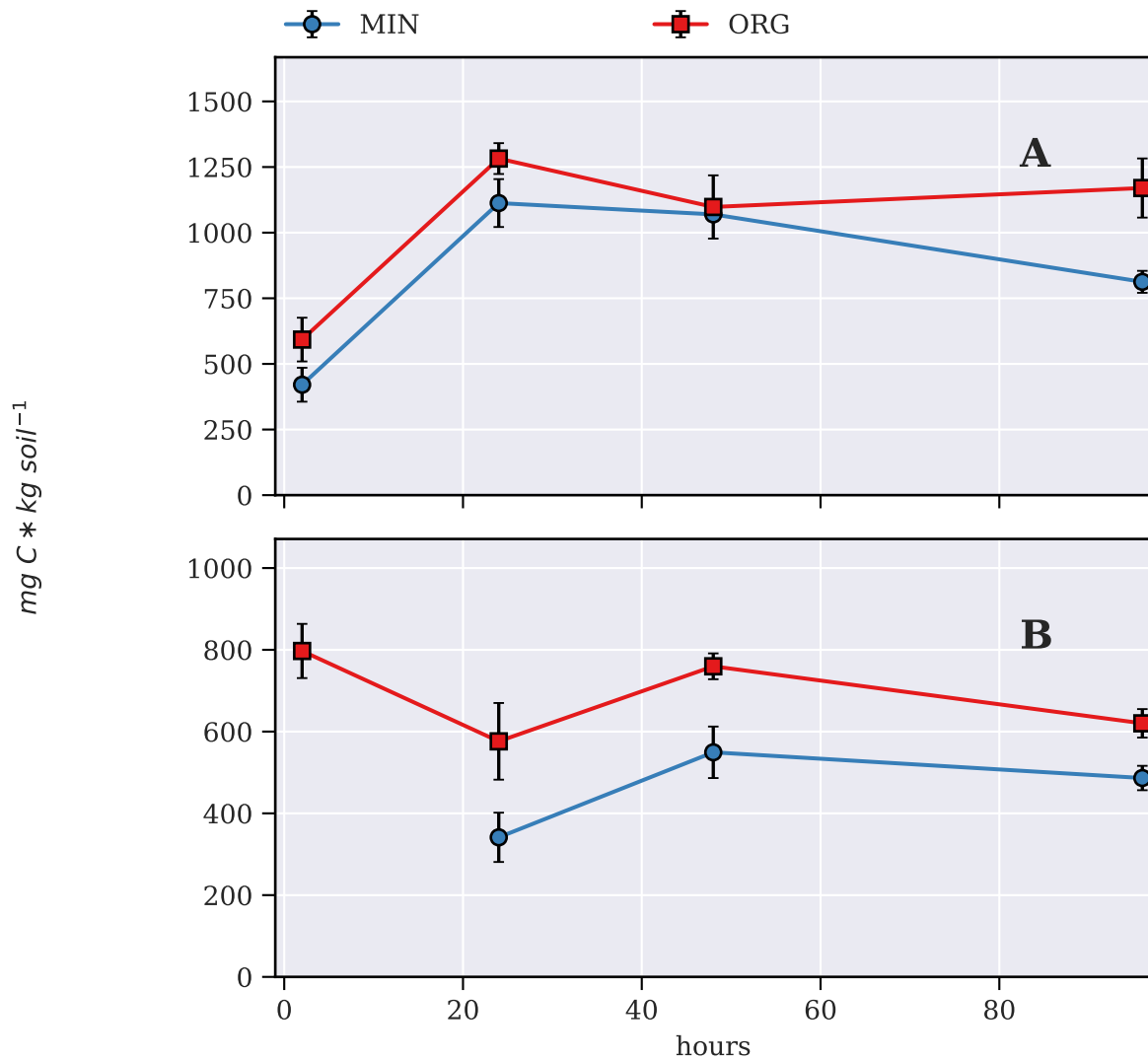


Figure 9: MBC in STR (A) and KWC (B) amended samples

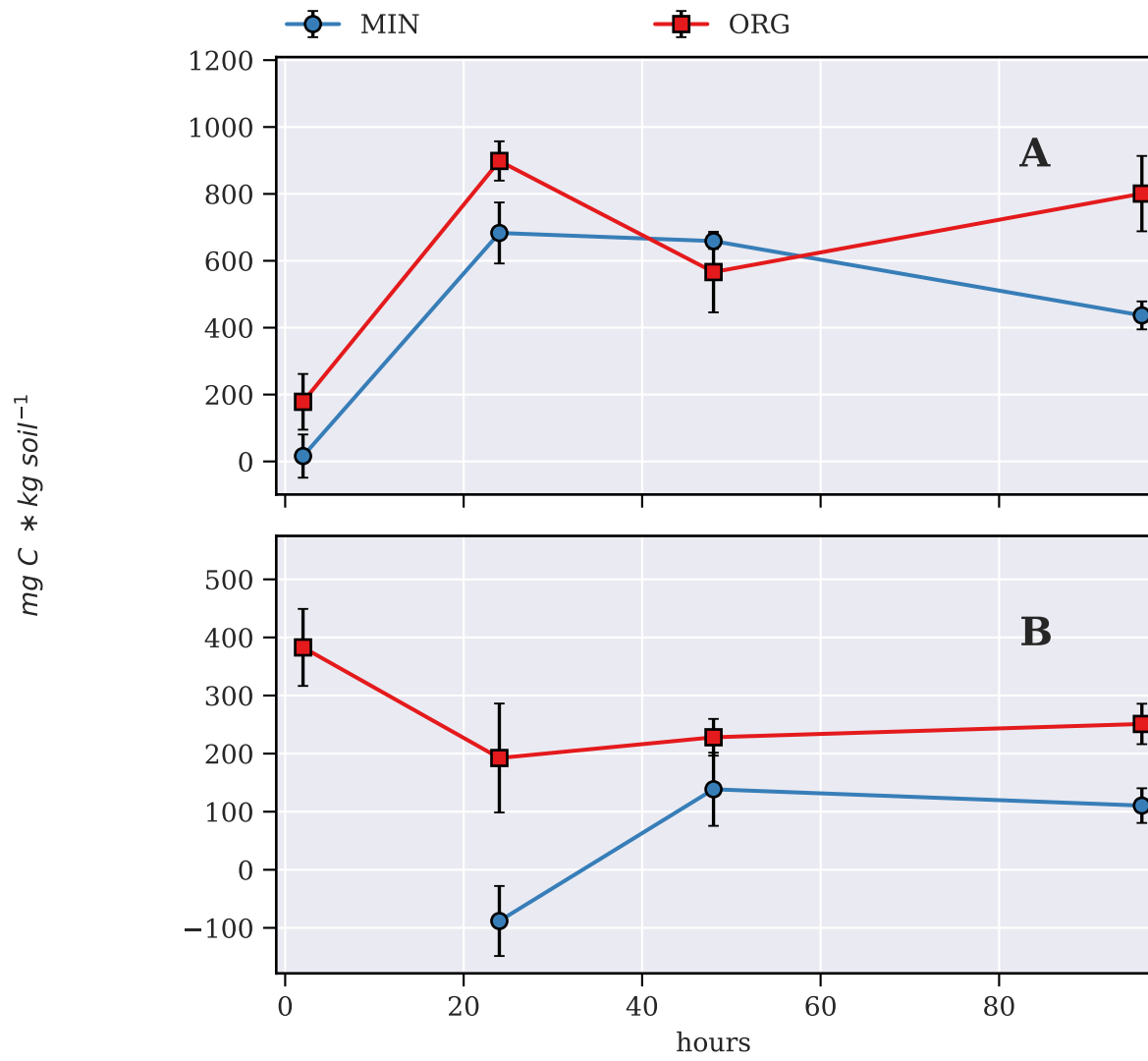


Figure 10: control normalized MBC in STR (A) and KWC (B) amended samples

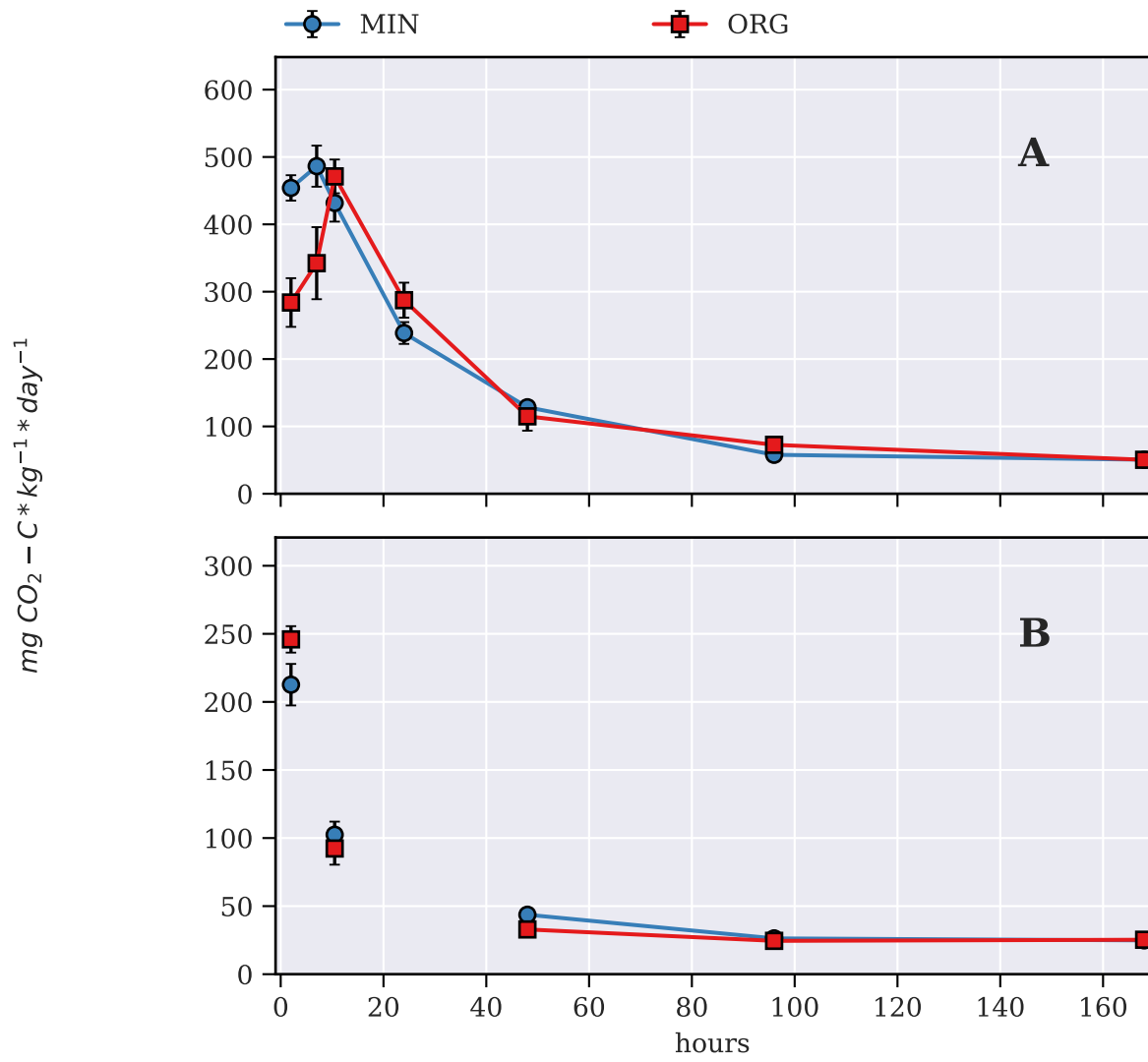


Figure 11: CO_2 in STR (A) and KWC (B) amended samples

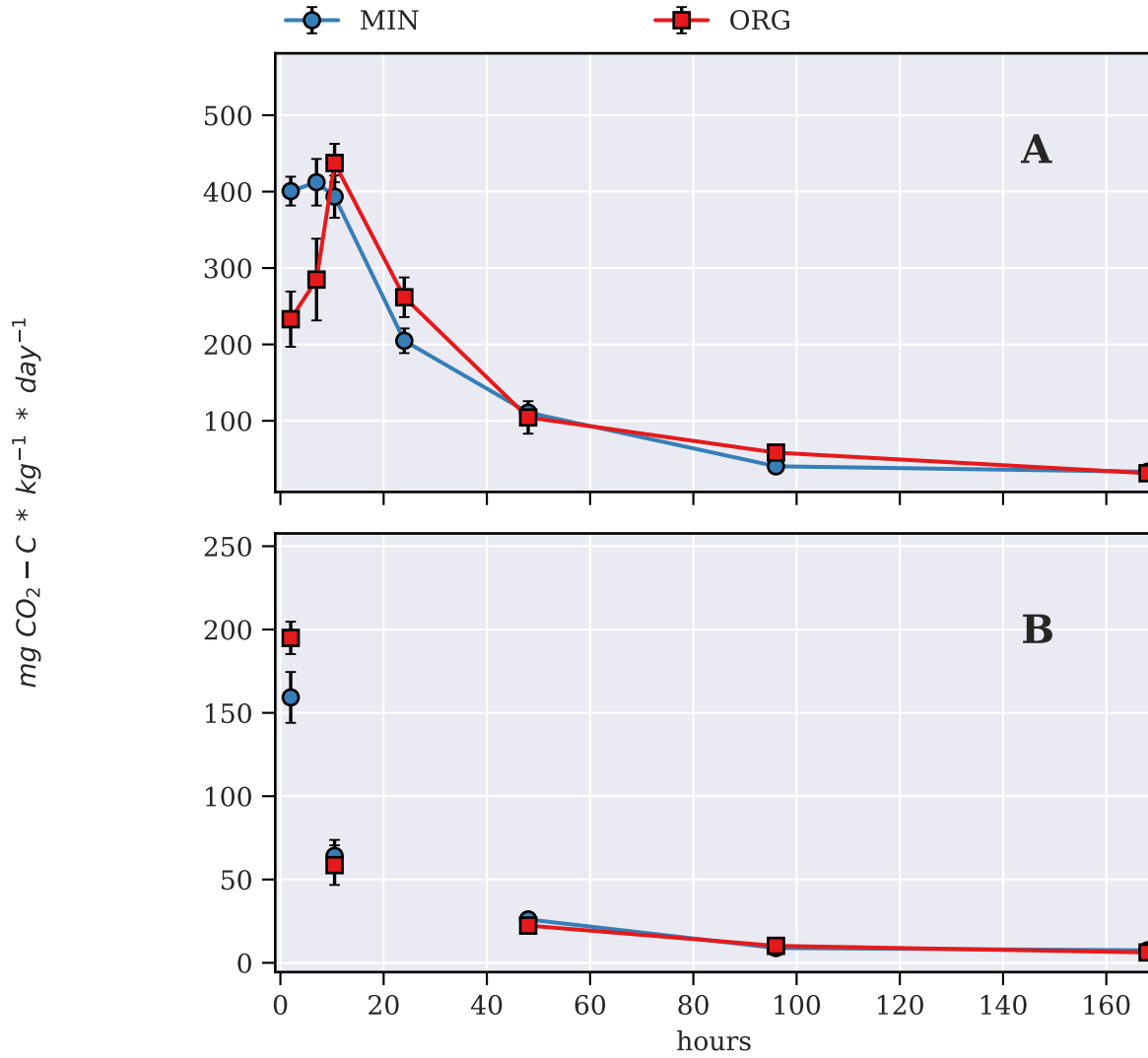


Figure 12: control normalized CO_2 in STR (A) and KWC (B) amended samples

WEOC

similar to microbial biomass and respiration, high Cn.WEOC (Fig 14) values were observed for all treatment combinations in the first sampling. STR amended samples had higher Cn.WEOC than KWC amended samples, with values ~4 and ~8 times higher than control values in Org+STR and Min+STR respectively, compared with ~2 and ~4 times higher than control in Org+KWC and Min+KWC respectively. WEOC dynamics (Fig 13) presented a general pattern in all samples, in which the initial increase in WEOC was followed by substantial decline, reaching a minimum level after 48 h and then increasing to varying degrees, depending mostly on STT. Notably, the decline in WEOC following initial increase was much stronger in STR compared to KWC in the first 24 h, and the overall decrease, in terms of the absolute difference, between the 2h sampling and the 48 h sampling was considerably larger in STR. Additionally, in the last 48 h, KWC samples had increased WEOC levels to values close to initial values, while the corresponding increase in STR was relatively much more limited, with final nor.WEOC values less than %30 percent that of initial values. As mentioned earlier for WEOC dynamics of control samples, the WEOC dynamics across the incubation period seem to inversely fit with those of MBC and this observation is made even clearer when considering the variability between the two STTs in this regard. For example, it can be noticed, that in KWC amended samples, MBC levels had seen very little changes in the first 24 h corresponding to

the more moderate decrease in WEOC in these samples, compared with the parallel decrease in STR samples. Similarly, in the last 48 h, a limited increase of WEOC in STR amended samples, corresponds with high levels of MBC while a relatively much larger increase of WEOC in KWC is accompanied by decreasing levels of MBC in these samples.

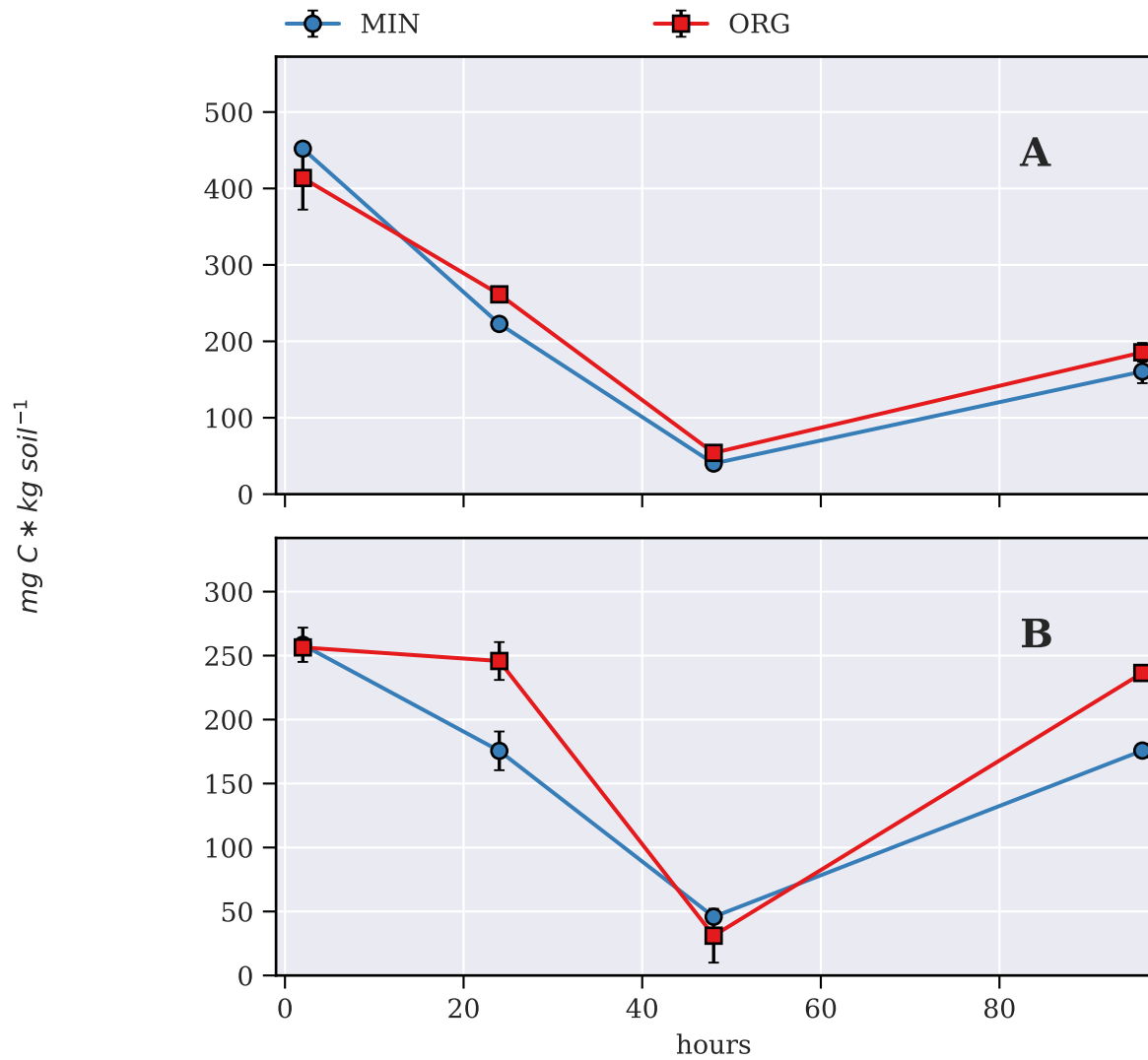


Figure 13: WEOC in STR (A) and KWC (B) amended samples

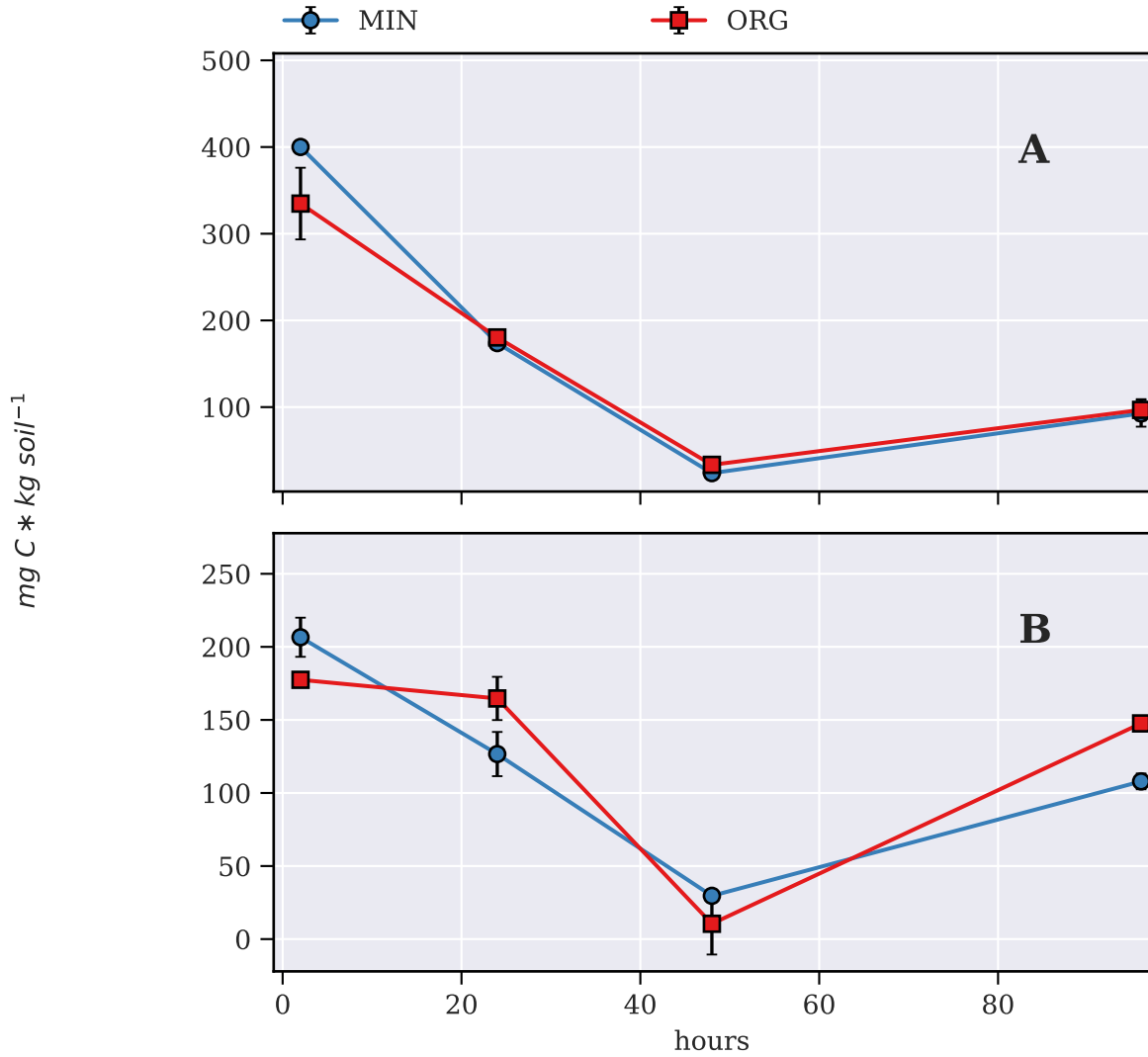


Figure 14: control normalized WEOC in STR (A) and KWC (B) amended samples

HWEC and HWES-C

In contrast with control samples, where HWEC and HWES levels differed significantly between LTTs during the entire incubation, only few occasions showed significant differences between the two LTTs in STR or KWC amended samples, when results are normalized to control (Fig 16 and 18). This may reflect a stronger effect of STTs over the effect of LTTs. In Org, KWC and STR had caused relatively small initial increases in Cn_HWEC and Cn_HWES-C, while Min amended samples had seen increases of 67 and 95 % over control samples in KWC and STR amended samples respectively. In KWC amended samples, both Org and Min samples had seen a reduction of HWEC (Fig. 15 B) down to levels below those of control samples after 48 h of incubation and these reductions were followed by an increase back to levels comparable to initial values. As observed for control samples, an almost constant ratio of HWES-C-to-HWEC was observed in all STT-LTT combinations, excluding the 48h sampling which saw a sharp increase in this ratio for KWC amended samples (in both LTTs). Although the HWES-C-to-HWEC ratio remained mostly constant, it had slightly increased under KWC and even more so under STR.

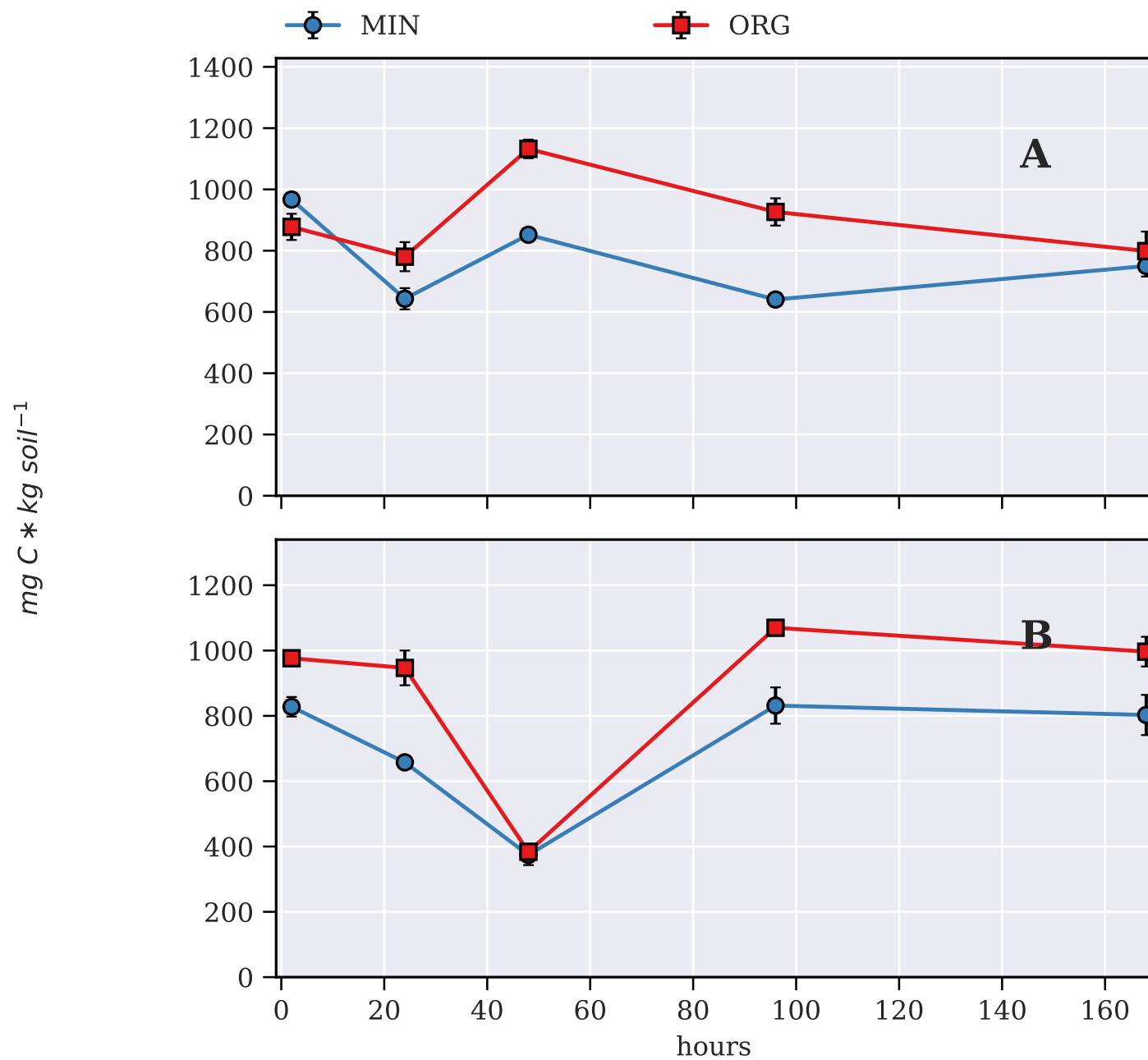


Figure 15: HWEC in STR (A) and KWC (B) amended samples

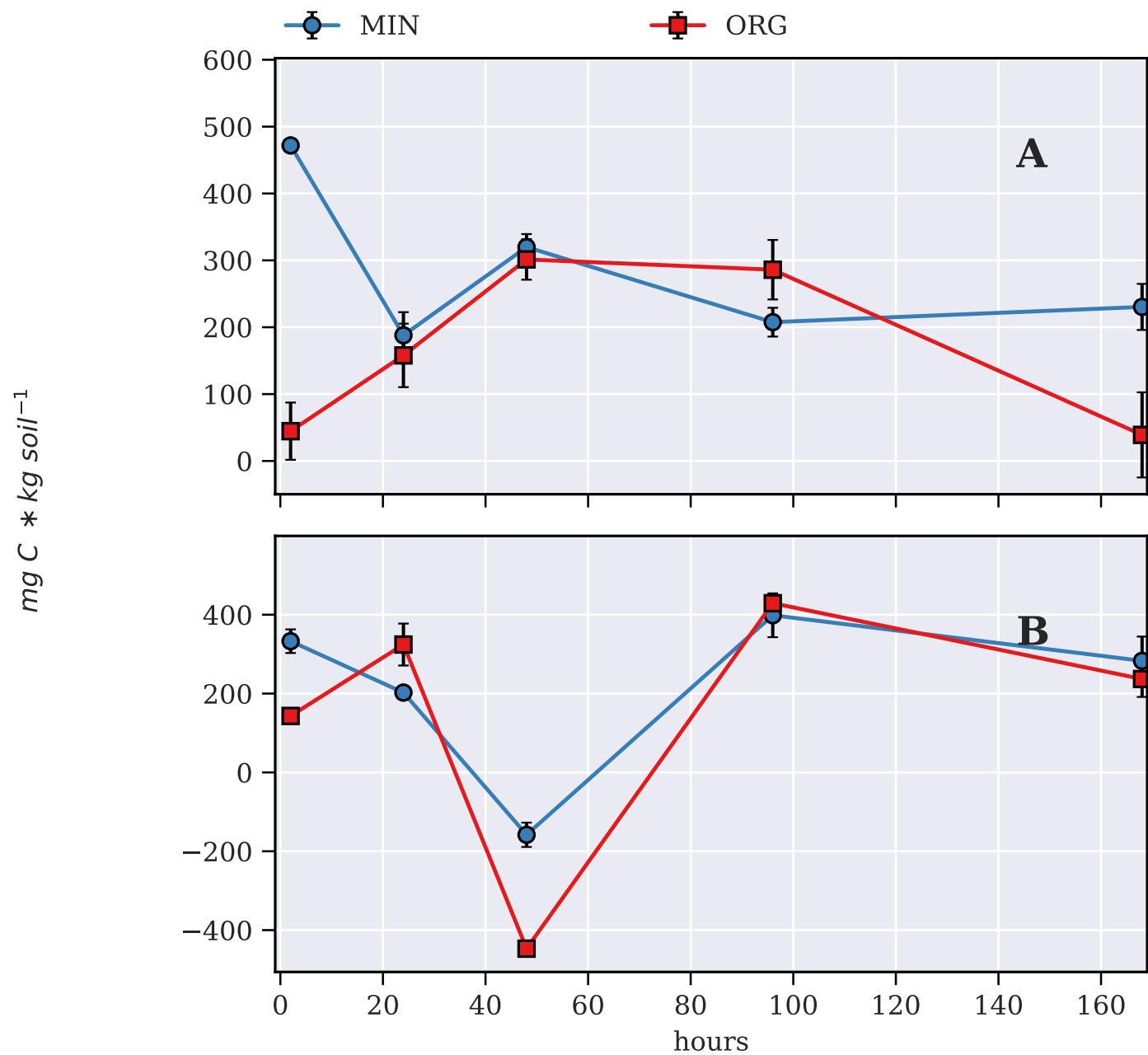


Figure 16: control normalized HWEC in STR (A) and KWC (B) amended samples

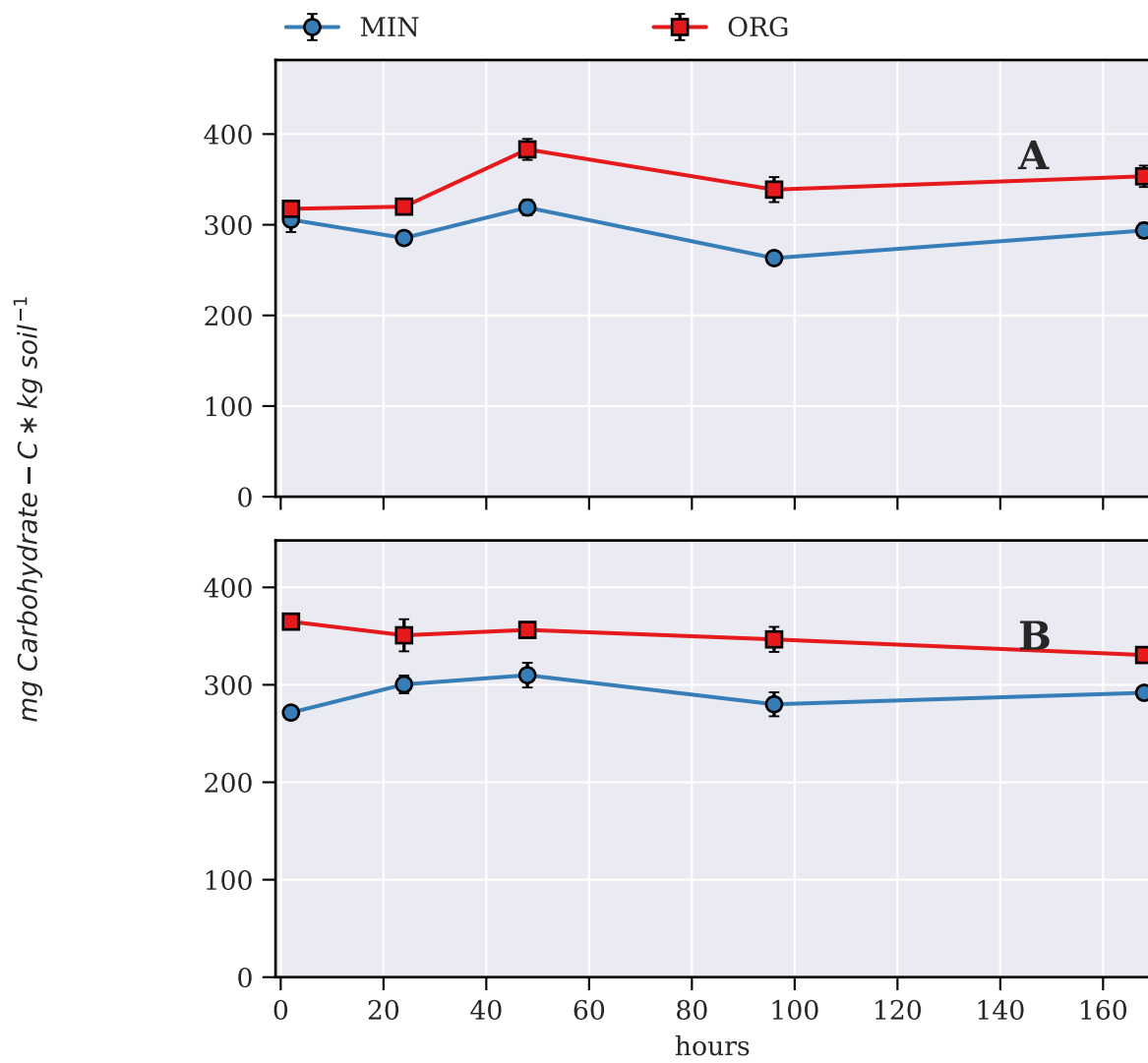


Figure 17: HWES-C in STR (A) and KWC (B) amended samples

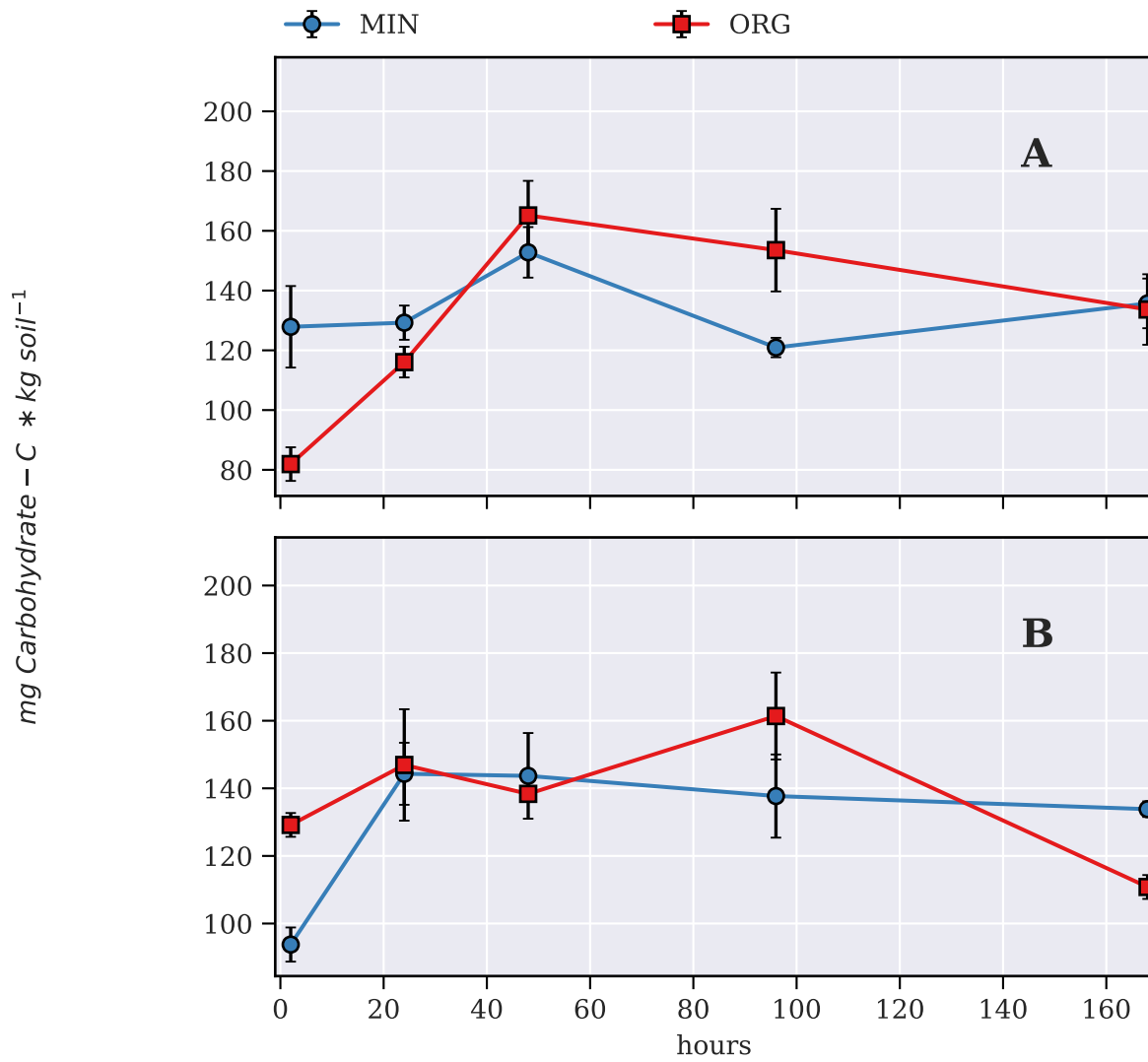


Figure 18: control normalized HWES-C in STR (A) and KWC (B) amended samples

Ergosterol

In STR amended samples (Fig. 19, A), Ergosterol levels rose sharply during the first 96h in both LTTs and remained high up to the end of the incubation. Contrarily, in KWC amended samples (Fig. 5, B), no significant changes were observed throughout the incubation. In STR amended samples, despite the significant increase in ergosterol concentration, no significant increase in Erg-to-MBC (Fig. 20 A) over the corresponding values in the control samples, was observed. This was similarly observed for KWC amended samples (Fig. 20 B).

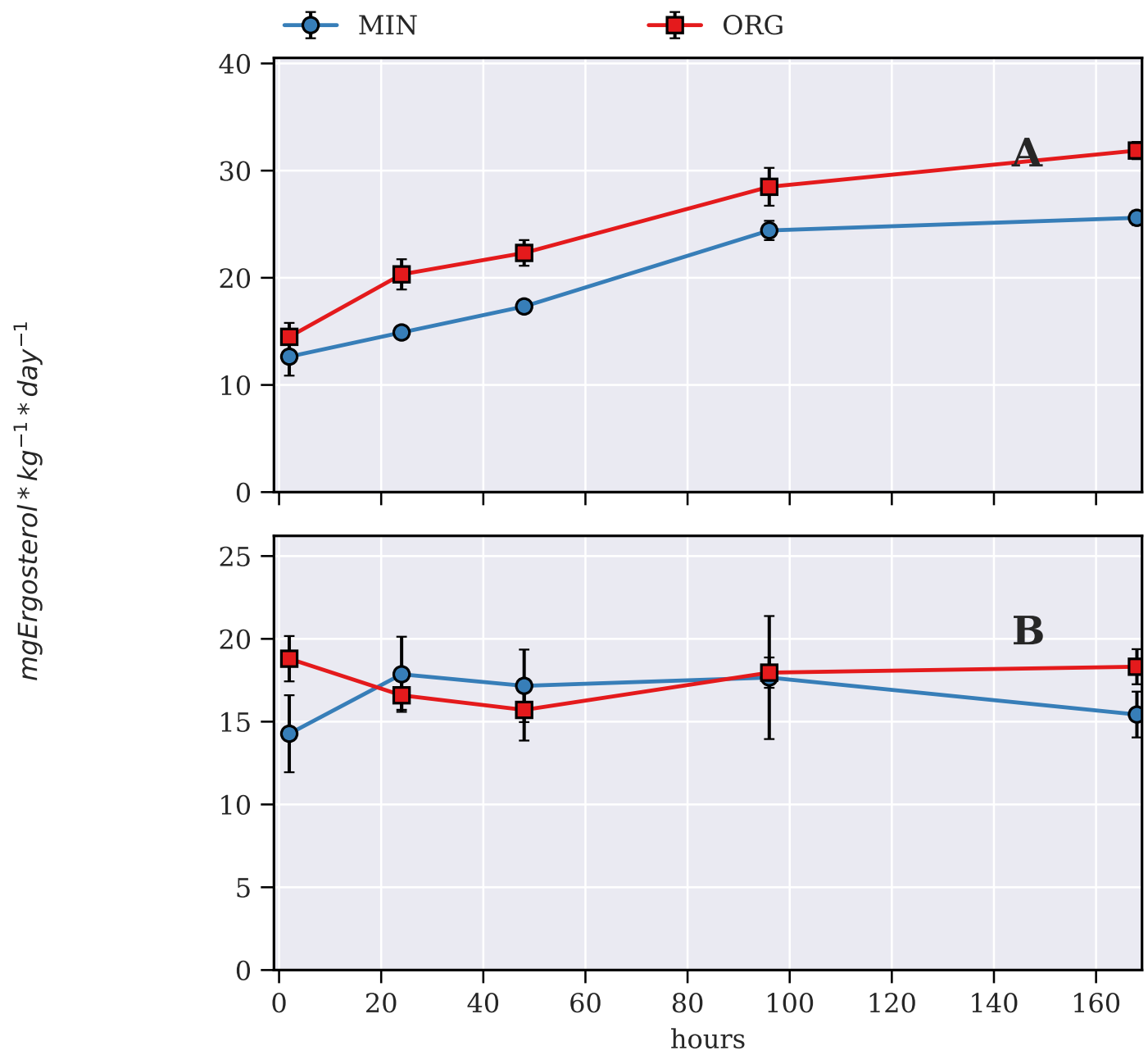


Figure 19: Ergosterol in STR (A) and KWC (B) amended samples

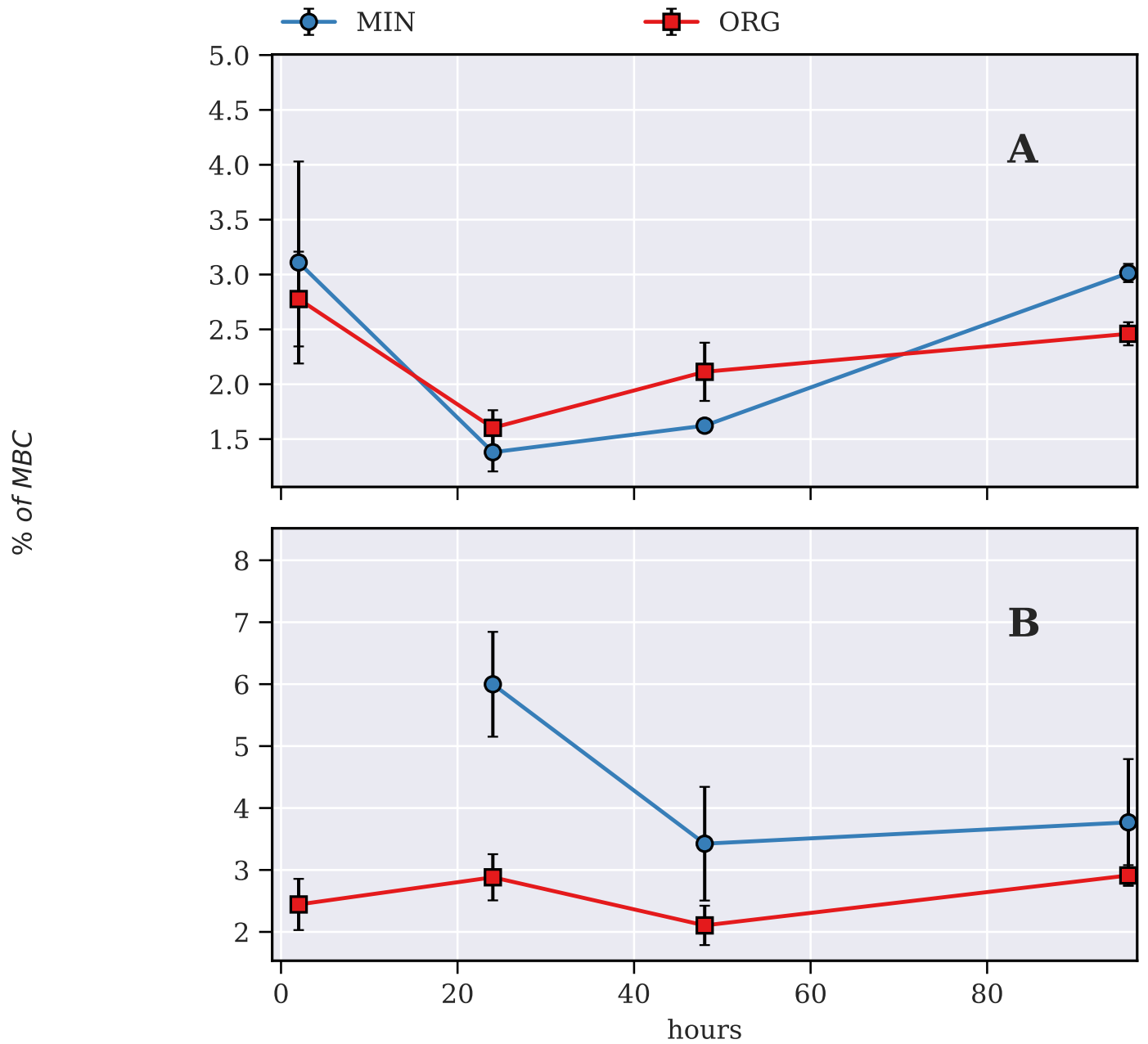


Figure 20: Ergosterol-to-biomass in STR (A) and KWC (B) amended samples

1

0.2 Incubation with MRE solution

In the following section, results for soil parameters that did not include in-week samplings, were presented either as bar plots or as tables. weekly MRE/water additions resulted in large in-week fluctuations for the following, frequently sampled for, soil features: MBC, Resp and WEOC (detailed description follows). the remaining soil parameters were only sampled at day 0 and every week end. plotting these data sets as connected lines implies a known variation for the dynamics of between sampling events which is not necessarily the case and therefore a bar plot or table representation was preferred.

0.2.1 SOM properties in non-amended soil samples

The first main objective for this work was to establish a reference point for SOM properties in the three LTTs and evaluate the effect of 5 years of different management histories on the short term dynamics of SOM properties as well as their average baseline values in non-amended samples.

short-term dynamics

the non-amended samples received distilled water as a control for MRE treated samples (in equivalent volume = 1ml per week). each water addition had raised the soil water content by roughly 10% of WHC from 50 to 60% WHC.

The first water addition was followed by sharp increases in MBC , RESP and WEOC in all three LTTs (Figures 21, 22 and 23 respectively). Strong pulses of CO₂-Respiration were also observed after the 2nd and 3rd water addition. Considerably smaller increases were observed in MBC between days 8-10 and between days 14-15 which seem to have been also related to water additions. These increases following water additions were not observed in WEOC (except for the first water addition).

Regardless of these pulses, a general trend was observed in the above mentioned parameters, entailing high values during the first part of the incubation (few days to one week depending on the parameter) followed by a decrease and then a steadying of values in later weeks. These similar patterns suggest a connection between microbial growth and activity and the concentrations of WEOC.

MBC had increased by roughly 10 fold in all three LTTs during the first 24 h. Subsequently, MBC levels dropped rapidly, reaching levels comparable to initial values in all three LTTs by the 8th day. from then on, another, slower and more limited in size increase in MBC was observed for all three LTTs, leveling off in the range of 221-464 $mg * kg\ soil^{-1}$, by the 10th day. A small but significant increase was again observed during the 14th day (immediately after the 3rd water addition) peaking in at 275-564 $mg * kg\ soil^{-1}$ by day 15 and subsequently decreasing slowly to reach values of between 200-300 $mg * kg\ soil^{-1}$ by the end of the incubation.

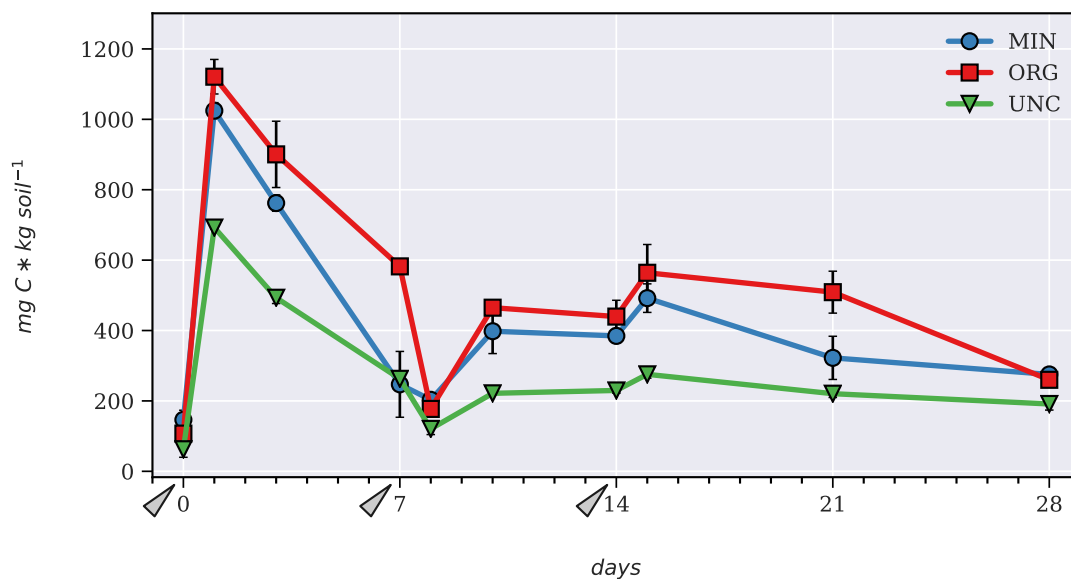


Figure 21: MBC in non-amended samples

A sharp pulse of CO₂ respiration was observed following each water addition. Respiration rates rose sharply in the hours following each water addition, peaking after 4h in the 1st and 2nd water additions and after 2h in week 3 (In week 3, no sampling was performed between 2-12 h after water addition). 4 hours after the first water addition respiration rates were increased by 30% or more, over the value for 2 h (first sampling), depending on LTT. After A ~50% decrease in the next 8 h, rates were mostly increasing steadily until the next water addition. The 2nd week saw another, even sharper pulse of respiration, after which respiration rates were constantly decreasing. The 3rd week, similarly saw a sharp increase and then decrease in the first 10 h after water addition and respiration rates subsequently fluctuated within a constant range until the end of that week.

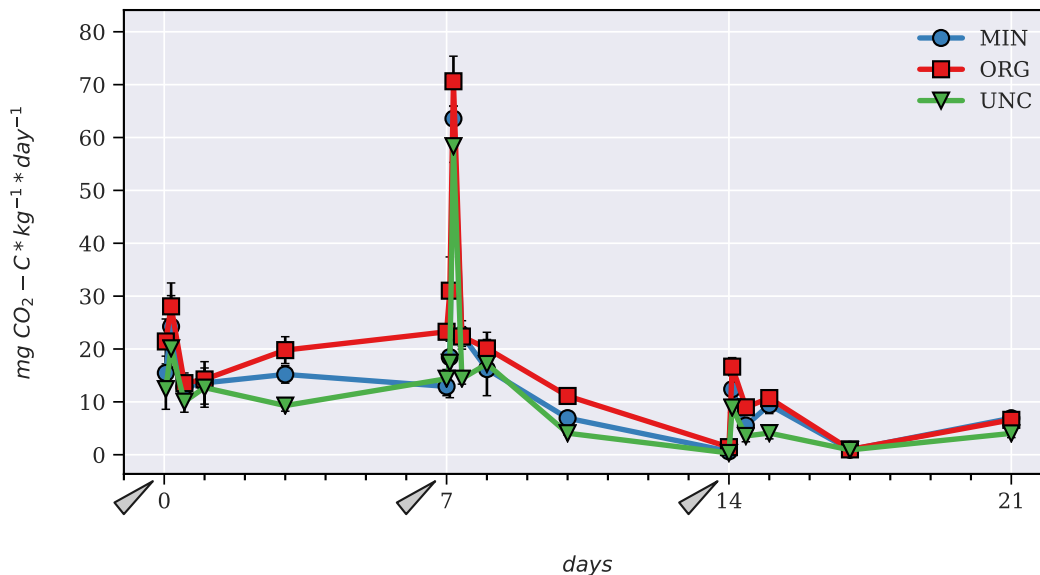


Figure 22: CO₂ Respiration in non-amended samples

Similar to the dynamics of microbial growth and respiration, WEOC levels had sharply increased in the first 24 h of incubation (by 300% in MIN and UNC and 400% in ORG), declining considerably during the first half of the 2nd week, and then increasing in the 2nd half and subsequently presenting steady values in the last 2 weeks. In contrast, we did not observe any increases following the 2nd and 3rd water addition, as we did with the dynamics of MBC and particularly those of RESP.

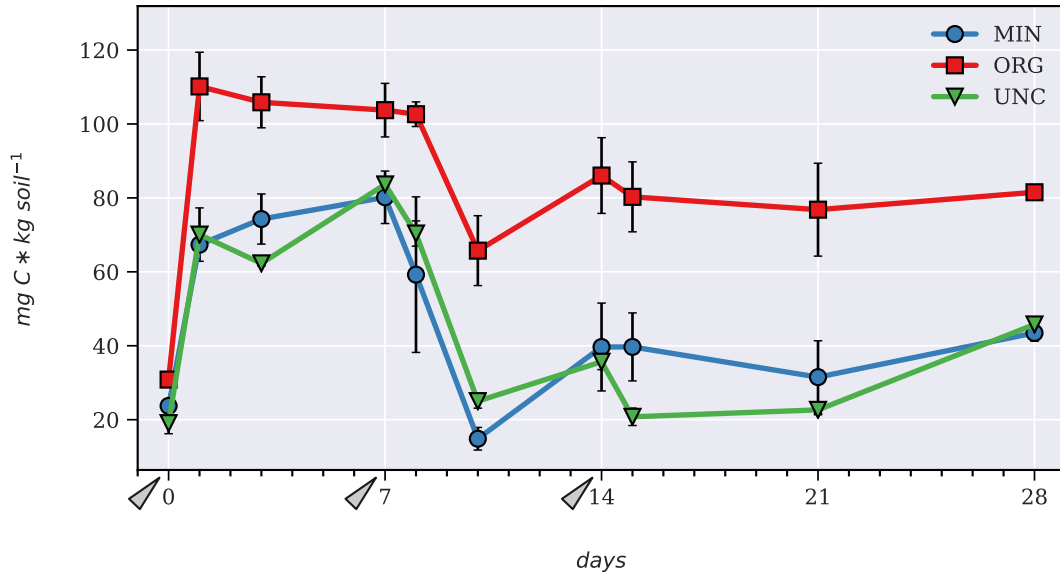


Figure 23: WEOC in non-amended samples

HWES (Fig. 24), in contrast with WEOC, showed only slight changes during the incubation, mostly restricted to the first half of incubation, in which a significant decrease (particularly in MIN and UNC) and then increase back to levels similar to initial values, was observed. HWES remained practically unchanged during the 2nd half of the incubation. Arguably, HWES samplings were limited to the first and last day of each week and it is possible that more pronounced changes would have been recorded for in-week samplings, as for example, was observed for MBC in the first few days of incubation or for WEOC on day 10. Nonetheless, weekly changes in MBC and WEOC were far more considerable compared with those in HWES, with percent changes in the range of hundreds on the 1st week (MBC and WEOC) and 2nd week (WEOC) (Figs. 21 and 23 for MBC and WEOC respectively).

Interestingly, the period where HWES levels decreased (1st week), correspond to a large extent, with the period of increased MBC, CO₂-Resp and WEOC.

Aggregate stability (Table 1), measured on days 0, 14 and 28, generally decreased throughout the incubation period, except in ORG in the 2nd half of the incubation.

Effect of management history in non-amended samples

Overall, the level of SOM parameters observed for control samples throughout the incubation where in the following order, *ORG* > *MIN* > *UNC*, However this pattern was somewhat inconsistent in some of the parameters. HWES values (Fig. 24) for ORG were significantly and substantially higher than for the two other LTTs on every sampling day and WEOC (Fig. 23) values significantly differentiated ORG from the two other LTTs on 6 out of 10 sampling events. A similar trend is true also for MBC (Fig. 22) concentrations and RESP (Fig. 22) although differences between ORG and MIN soil are mostly statistically insignificant and the results are generally not as clear. As mentioned above, MIN had mostly higher values than UNC for most parameters throughout the incubation but this was clear only for HWES concentrations, where differences between the two soils are statistically significant on every sampling day.

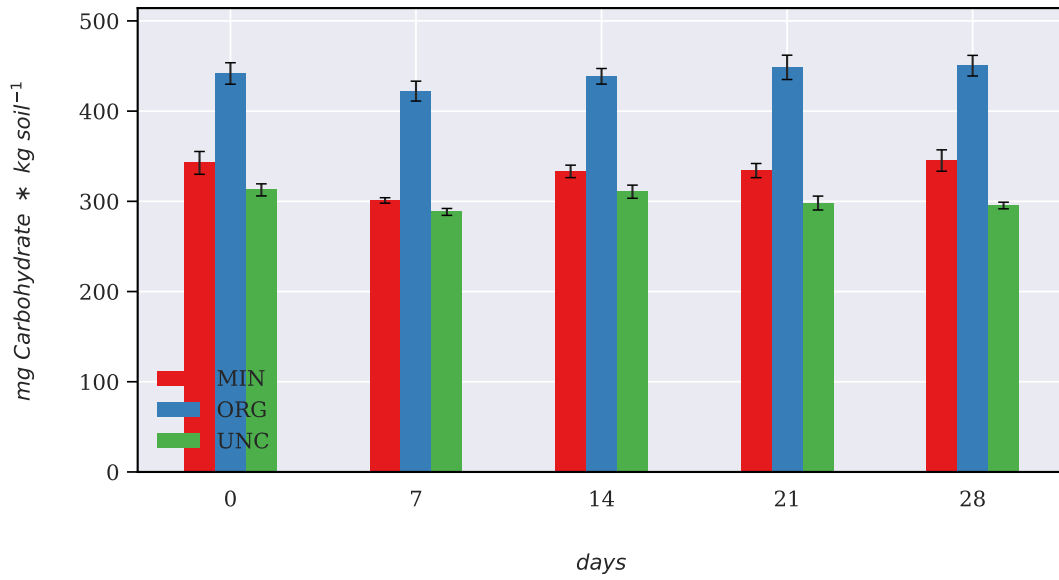


Figure 24: HWES in non-amended samples

Considerable differences were observed between LTTs in cumulative respiration (Fig 25), in the 2nd and particularly in the 3rd week, with the same order generally observed for other parameters, that is ORG > MIN > UNC. Total cumulative respiration after 3 weeks of incubation was 154, 199 and 261 in UNC, MIN and ORG respectively.

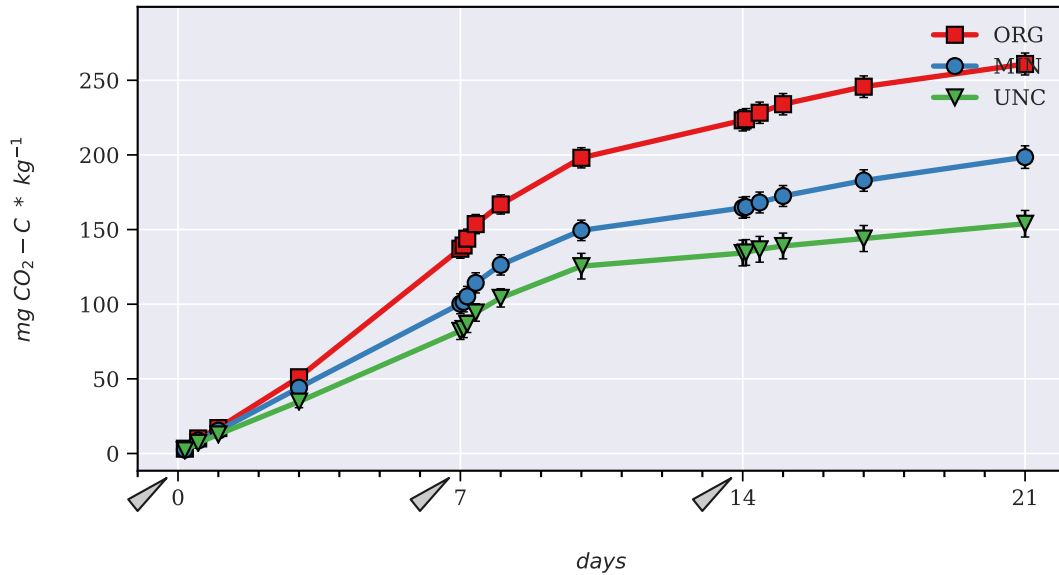


Figure 25: Cumulative respiration in non-amended samples

as mentioned earlier, the %WSA (Table 1) generally decreased during the incubation. This was particularly notable in ORG and MIN during the first half of the incubation, with a decrease of more than 5.5% in both LTTs. A much more limited decrease was observed in UNC during that same period. The total decrease in MIN was roughly double the decrease observed for UNC, while ORG had actually sustained the lowest total decrease in WSA, despite large initial decrease.

Strong AS decreases in the first half of the incubation in Org and Min, corresponded to strong microbial activity, compared with the second half of the incubation which showed much smaller decreases (and even an increase for Org) and was incidentally also characterized by a more

limited microbial growth and seemingly also microbial respiration (data for the last week not available).

Table 1: Aggregate Stability in Control samples

soil days	MIN	ORG	UNC
0	19.33 ± 0.89	18.90 ± 0.89	10.42 ± 0.81
14	13.76 ± 1.63	13.31 ± 1.59	9.44 ± 1.69
28	12.85 ± 0.94	17.01 ± 0.68	6.98 ± 0.35

A slight increase in Erg (Fig. 26) was observed for Org and Min during the incubation period while UNC saw an overall slight decrease despite considerable increase in the 1st week . In contrast, Erg as a percent of MBC (Fig. 27) showed a clear reduction in Org and UNC during the 28 days incubation, with most of this increase occurring in the first half of the incubation. Min samples had seen no significant change in %Erg of MBC in the course of incubation.

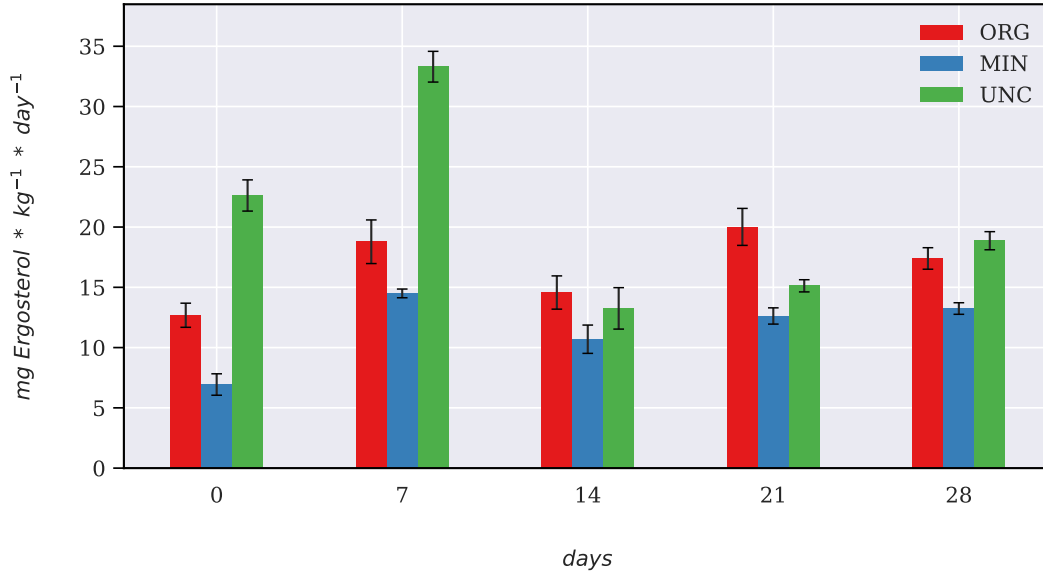


Figure 26: Ergosterol concentration in non-amended samples

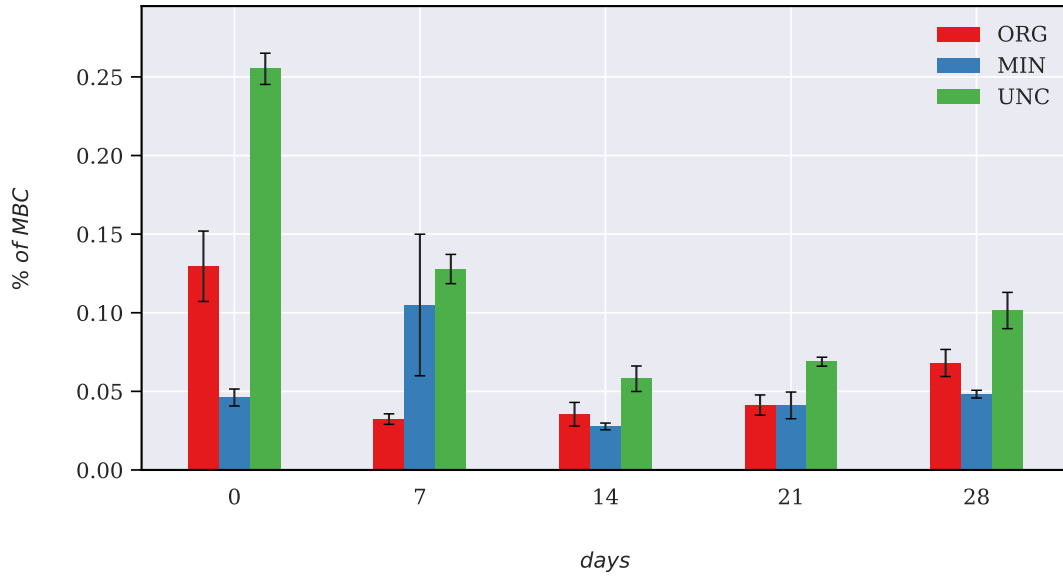


Figure 27: Ergosterol-to-Biomass ratio in non-amended samples

Baseline

Table 2 shows average values of control samples across day 0 and every week end of the incubation for each LTT (a total of 20 replicates each). Sampling events between week ends were omitted here to eliminate the effect of water additions at the beginning of each week and provide a baseline value that will correspond as accurately as possible to a certain normal level for each parameter-LTT combination.

the general order of LTTs described above ($ORG > MIN > UNC$) for the 28 days dynamics in most soil properties, is similarly maintained when average baseline values are calculated, though significance between LTTs did differ. ORG had a significantly higher baseline value in all measured parameters except Erg compared with UNC, while only MBC, AS, and HWES were significantly different between MIN and UNC. Differences between ORG and MIN were significant in TOC, WEOC and HWES, while no significant differences were observed in MBC, CO₂-Resp and AS between these two LTTs (although all of these parameters were higher in ORG). The lack of significance between ORG and MIN in MBC and CO₂-Resp rates, probably reflects, to a large extent, the high degree of variability in the data, due to technical but mostly natural sources of variability. Statistical insignificance between ORG and MIN in AS is more likely to reflect actual closer similarity between these two LTTs, at least when compared with UNC. Differences in AS between ORG and MIN were relatively smaller than those in MBC and CO₂-Resp, errors were relatively smaller and the difference between UNC and the two other soils was roughly 40% the maximum AS value (ORG). Thus, it seems that indeed %WSA, at least for this aggregate size division ($> 250\mu m$), was similar in ORG and MIN.

to summarize, **our** results suggest that SOM pools in ORG were different from those of the two other LTTs, despite some of these differences not being statistically significant, while MIN and UNC may have been somewhat more similar to each other in terms of SOM properties than each one of them is similar to ORG. Nonetheless, some properties, particularly AS but also MBC and respiration, suggest a considerable distinction between MIN and UNC.

Table 2: baseline values of SOM related properties

	MBC	MBN	Resp	HWES	WEOC	AS	Erg	TOC	TON
ORG	385.87 a	49.70 a	10.43 a	440.27 a	75.79 a	16.69 a	16.69 b	1.71 a	0.15 a
MIN	279.68 a	50.29 a	6.81 a	331.23 b	43.71 b	14.95 a	11.60 c	1.11 b	0.09 c
UNC	200.28 b	32.72 b	6.79 a	301.01 c	41.36 a	8.81 b	20.63 a	1.08 a	0.10 b

0.2.2 Dynamics of SOM properties in MRE treated samples

The results below are presented with regard to the effect of long term soil management on the short term soil reaction to labile organic input (specific objective # 2).

Microbial Biomass Carbon

The first addition of MRE had a clear and similar effect on MBC in all three soils, as evidenced from the sharp increase of more than 2000 $mg * kg\ soil^{-1}$ that was recorded in all treated soils 24 hours after the addition (figure 28). This increase constituted a peak of MBC in the first week (as well as the entire incubation) after which MBC dropped by more than a 1000 $mg * kg\ soil^{-1}$ by the beginning of the 3rd day of incubation. This decrease continued in the next few days, reaching average MBC levels of between 500 and 1000 $mg * kg\ soil^{-1}$ in the MRE treated LTTs by the end of the first week. Questionable data collected on the 8th day of incubation (24 h after the 2nd pulse) and missing data from the 17th day of incubation (72 h after the 3rd pulse) make it hard to assert the precise day of peak MBC in the 2nd and 3rd week. Nonetheless it is highly plausible that a similar trend as in the 1st week of incubation occurred in these two subsequent weeks in all soils, judging from the more reliable existing MBC data and from RESP data. Assuming that the dynamics of MBC indeed took on the same shape in the 2nd and 3rd week as in the 1st week, it can be seen that every MRE input is followed by a pulse of microbial growth reflected in the sharp increase and then decrease in MBC and RESP.

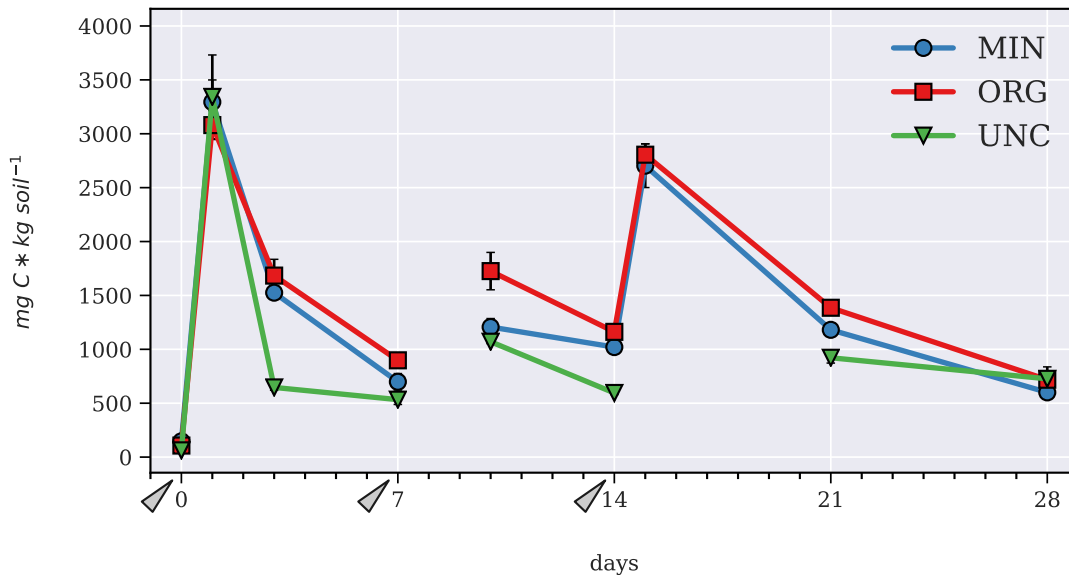


Figure 28: MBC in MRE amended samples

the weekly microbial growth in all three LTTs (Table 3) was highest during the 1st week and was then significantly and strongly reduced in the 2nd week, particularly for ORG and UNC. the 3rd week saw higher growth than in the 2nd week for ORG and UNC but not for MIN. the last week, in which no input was applied, saw substantial negative growth (net decrease in MBC). weekly growth was significantly higher in ORG compared with the two other LTTs in the first week. conversely, ORG saw the strongest negative growth in the last week though this was statistically non-significant. **Similar trends as described above are observed for net MBC.**

Table 3: weekly microbial growth and cumulative respiration

soil	week feature	1	2	3	mean
MIN	cumulative respiration	839.477753	1097.370139	630.810139	855.886010
	microbail growth	552.405700	321.842334	160.213889	344.820641
ORG	cumulative respiration	921.424907	1087.323878	893.015413	967.254733
	microbail growth	790.758319	264.123434	223.559873	426.147209
UNC	cumulative respiration	795.478156	1224.330781	454.070439	824.626459
	microbail growth	470.433481	61.890408	326.782025	286.368638

similar or very close values of MBC were observed for ORG and MIN soils throughout the incubation with ORG exhibiting slightly higher values on most days (significant on day 8 and 21), while MBC dynamics for UNC diverged, at least to some extent from that of the two other soils with significantly lower values observed on the 3rd and 10th day of incubation (3rd day of 1st and 2nd week respectively) as well as on the 14th and 21st day of incubation (end of 2nd and 3rd week respectively). Interestingly, by the end of the incubation, absolute MBC values converged to a similar level of roughly $700 \text{ mg} * \text{kg soil}^{-1}$ in all treated soils. compared with pre-incubation values, this value is equivalent to an increase of roughly $500\text{-}600 \text{ mg} * \text{kg soil}^{-1}$ MBC in the treated soils after 4 weeks of incubation.

Microbial Respiration

Similar to MBC, CO₂-Resp dynamics (Fig 29) presented a weekly pulse shortly after each MRE addition, albeit, these pulses were much closer in time to MRE addition. In the first two weeks, respiration rates peaked two hours after MRE addition at an average (across LTTs) of 1183 and 623 $\text{mg CO}_2\text{-C} * \text{kg}^{-1} * \text{day}^{-1}$ respectively, while the last week saw a considerably smaller peak at 246 $\text{mg CO}_2\text{-C} * \text{kg}^{-1} * \text{day}^{-1}$, which was recorded 10 h after MRE addition (no sampling occurred between 2-10 h). The high peak respiration rate in the first week, was followed by a general trend of sharp decrease leading to values close to the final value for that week after 3 days of incubation. The second week, unlike the first week, was characterized by a much smaller peak (roughly half the first peak) as well as higher rates in the following days, compared with a similar time period in the first week. These dynamics, entailed a considerably more flattened pattern of respiration in the second week. The third pulse was relatively similar to the 2nd pulse (2nd week) in the general pattern, compared with the first pulse, by having a much more limited peak respiration rate and relatively high rates in the next 24 h.

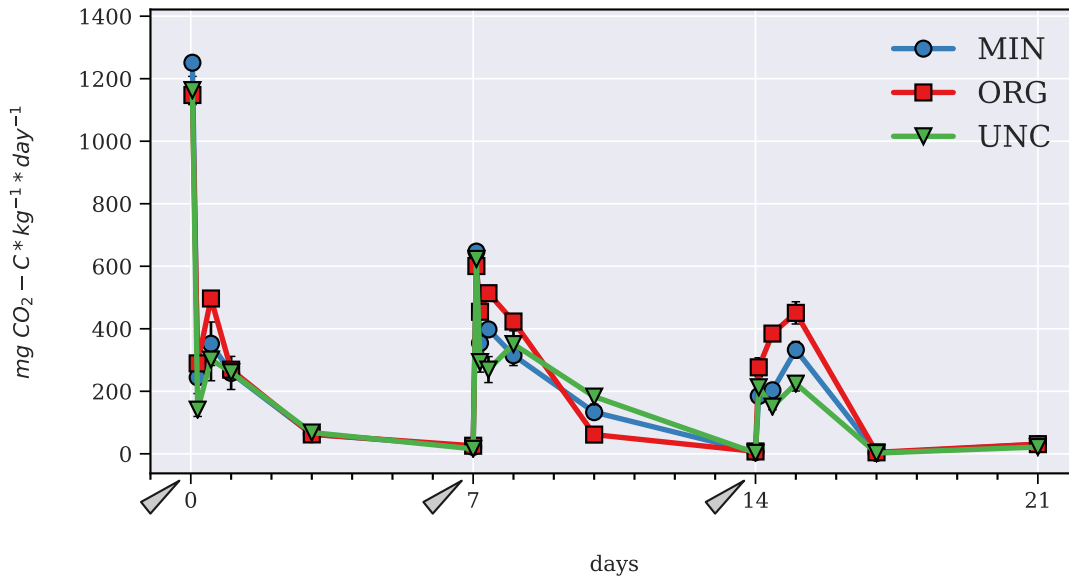


Figure 29: CO_2 respiration in MRE amended samples

the average weekly cumulative respiration for all three LTTs (Table 3) rose from $852\ mg\ CO_2 - C * kg^{-1}$ in the 1st week to $1136\ mg\ CO_2 - C * kg^{-1}$ in the 2nd week and then declined again to $659\ mg\ CO_2 - C * kg^{-1}$ in the 3rd week. these weekly values correspond to roughly half or less of the weekly portion of MRE-C ($2200\ mg * kg\ soil^{-1}$).

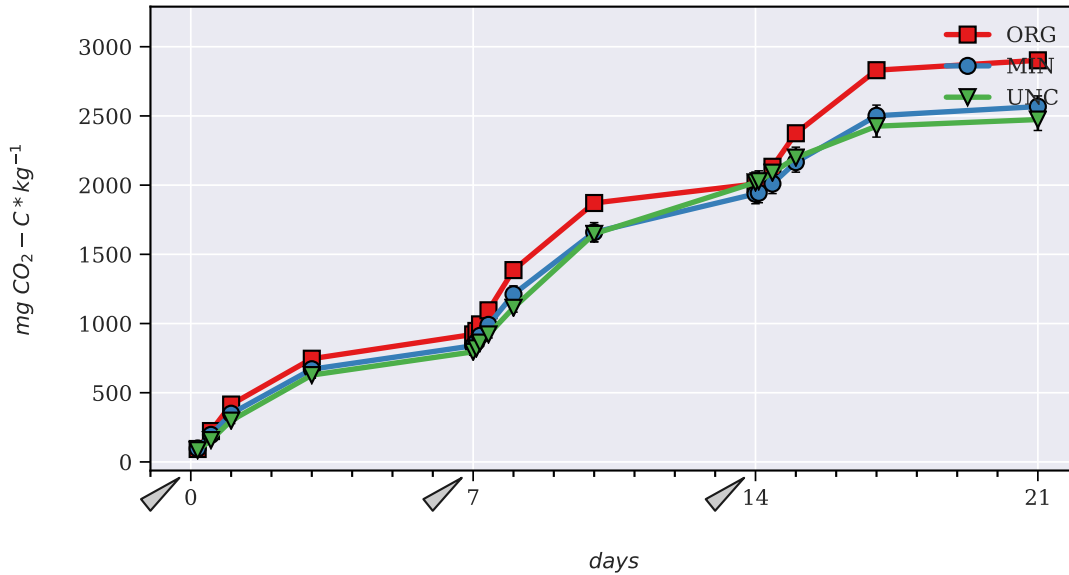


Figure 30: cumulative CO_2 respiration in MRE amended samples

Water Extractable Organic Carbon

Missing WEOC data on the 8th day of incubation prevent a definite description of UNC dynamics in the 2nd week and missing data on the 18th day prevents a detailed description of WEOC dynamics in the the third week for all three LTTs. For day 8, a presumed data point was added based on the trends observed in adjacent weeks. The dynamics of WEOC (Fig. 31) presented an interesting trend which differed significantly between LTTs. The first MRE addition saw a sharp increase of almost $1000\ mg * kg\ soil^{-1}$ in net WEOC after 24 h in UNC samples whereas only a relatively slight increase of 106 and $33\ mg * kg\ soil^{-1}$ was observed for MIN and

ORG respectively. This increase was almost entirely diminished by the 3rd day of incubation in all three LTTs and returned to values very close to the initial values after 7 days. These dynamics were similarly repeated in the 2nd and 3rd weeks, however, WEOC pulses following MRE input increased substantially between subsequent weeks in MIN and UNC. This meant that both the peak of WEOC pulse and its duration (I.e the time period before WEOC returned to baseline level) were increased, with peak WEOC in the second week standing at ~ 500 and $\sim \# \text{ mg} * \text{ kg soil}^{-1}$ for MIN and UNC (assumed) respectively, and these values almost doubled in the third week in MIN. In sharp contrast with these two LTTs, WEOC pulses after each MRE addition in ORG were considerably minor, with peak WEOC in the last week amounting to less than $150 \text{ mg} * \text{ kg soil}^{-1}$. These contrasting trends between LTTs, inversely correspond with the respective respiration trends in these LTTs. In the second week of incubation, and still more in the 3rd week, lower respiration clearly corresponded with high WEOC pulse as evident from comparing the rate of change in cumulative respiration with pattern of WEOC (Fig 31).

considering the intensity of these WEOC pulses immediately following MRE additions, and the above mentioned inverse relation with respiration rates, it seems highly likely that the these sharp increases in WEOC largely represent unprocessed substrate.

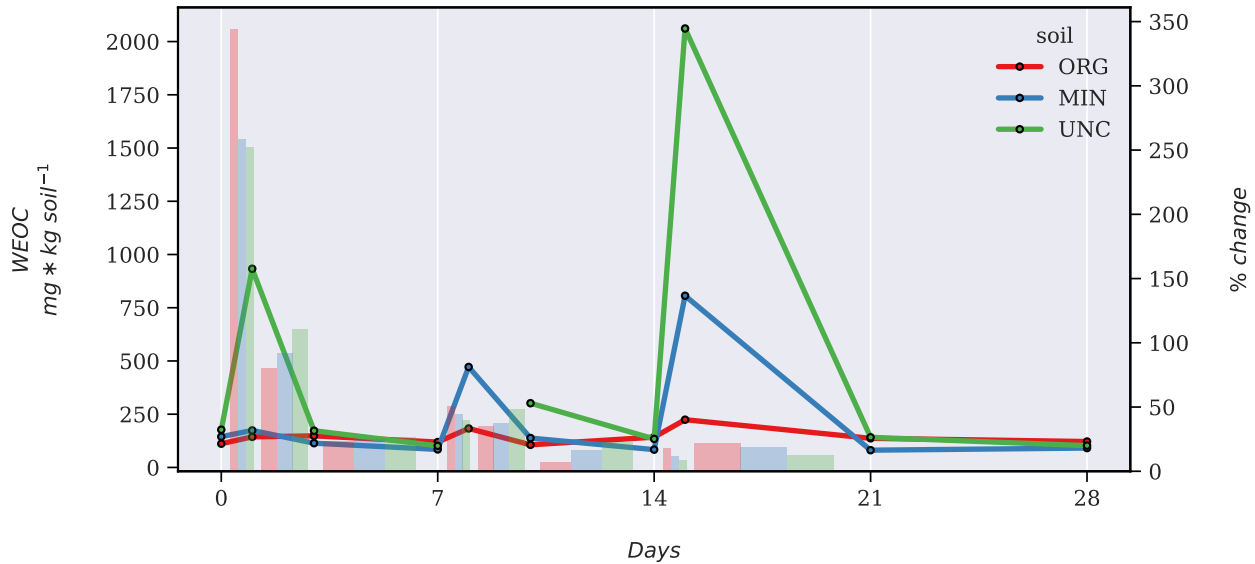


Figure 31: WEOC (left side y axis, solid lines) and % change in cumulative respiration (right side y axis, transparent bars) in MRE amended samples

Hot water extractable sugars

Throughout the incubation period, ORG maintained highest absolute values of HWES whereas on the final sampling date a comparable level of HWES (roughly $500 \text{ mg} * \text{ kg soil}^{-1}$) was observed for all treated soils (Fig 32). ORG had the highest HWES at the end of the incubation but this result was highly insignificant. UNC and MIN presented similar absolute values of HWES throughout the incubation. This probably reflects, at least to a certain degree their respective SOM stocks compared with ORG as HWES are often correlated with SOC. When these results are normalized to the control value (figure #), a different prospect of the HWES dynamics emerges, whereby during the first two weeks the three LTTs maintained similar values while the last two weeks saw a clear divergence between UNC and the two other LTTs. The first week saw an increase of $100 \text{ mg} * \text{ kg soil}^{-1}$ or less over control values in all three LTTs and the 2nd week saw a similar increase in all three LTTs. In contrast, during the third week, UNC saw a considerable increase of $\sim 50 \text{ mg} * \text{ kg soil}^{-1}$ while insignificant changes were observed for

ORG and MIN. the last week saw a very similar reduction of $\sim 50 \text{ mg} * \text{kg soil}^{-1}$ in MIN and UNC and roughly double that reduction in ORG. significant difference was observed between UNC and the two cultivated LTTs on the the last sampling event but not between ORG and MIN.

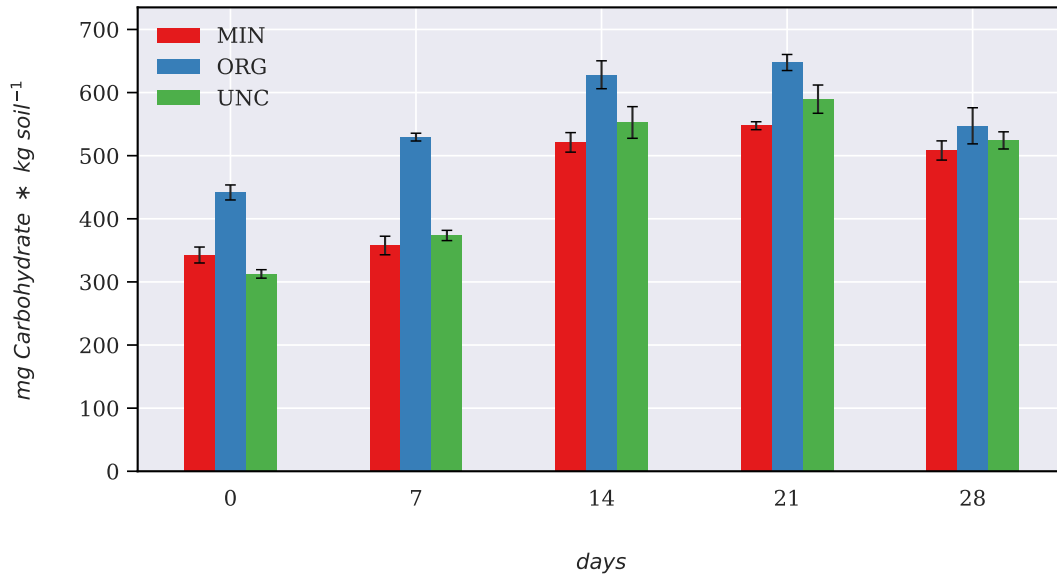


Figure 32: HWES in MRE amended samples

Microbial biomass nitrogen and C/N ratio

MBN values in MRE treated soils (figure 33) ranged between roughly 10 to $150 \text{ mg} * \text{kg soil}^{-1}$. MBN dynamics for ORG and MIN were similar in general trend. A sharp increase of roughly 7 fold was observed in first two weeks for MIN and ORG soil. In the 3rd week, increases were very small and the 4th week resulted in a slight decrease for MIN and a much larger decrease for ORG bringing the two LTT' to a similar value of between $80\text{-}90 \text{ mg} * \text{kg soil}^{-1}$. no apparent increase in MBN was observed For UNC in the 1st week. In the 2nd week an increase comparable to that of the two other soils was measured and from then on MBN levels stayed relatively steady, reaching a final value of $60 \text{ mg} * \text{kg soil}^{-1}$.

Microbial C-to-N ratio ranged from roughly 6 to 11 for the three soils, throughout the incubation (fig 34), with the highest significant value observed for UNC (11.03) after 3 weeks of incubation. An increase in microbial C-to-N ratio over initial value was visible on day 7, 14 and 21 for UNC and ORG while values for MIN stayed relatively steady, with only minor increases in these sampling days. The last week of incubation resulted in a decrease in normalized C-to-N ratio, especially for UNC and ORG. almost completely undoing the prior increases for MIN, while values for UNC remained relatively high (differences between MIN and UNC insignificant).

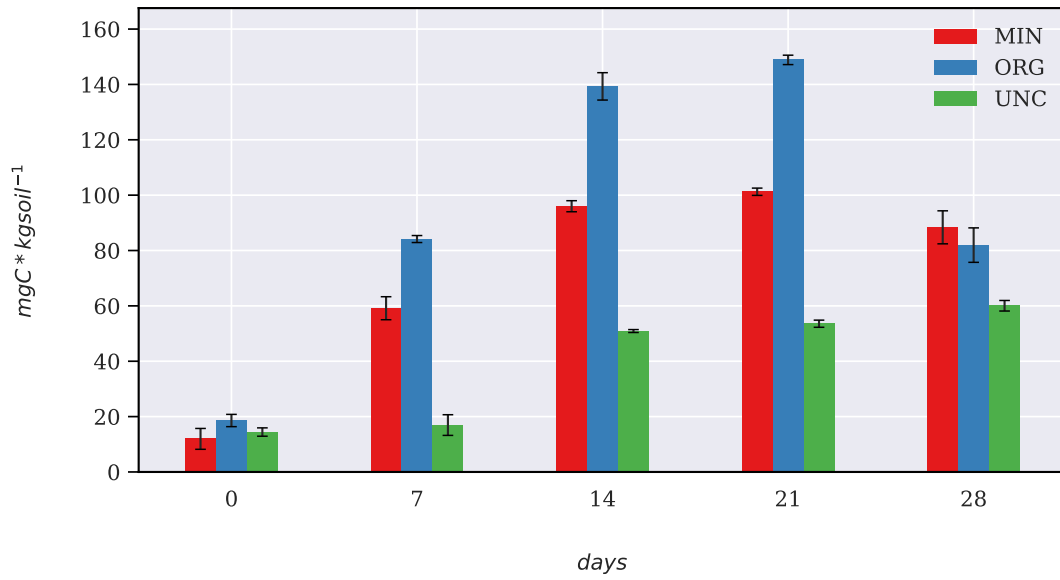


Figure 33: MBN in MRE amended samples

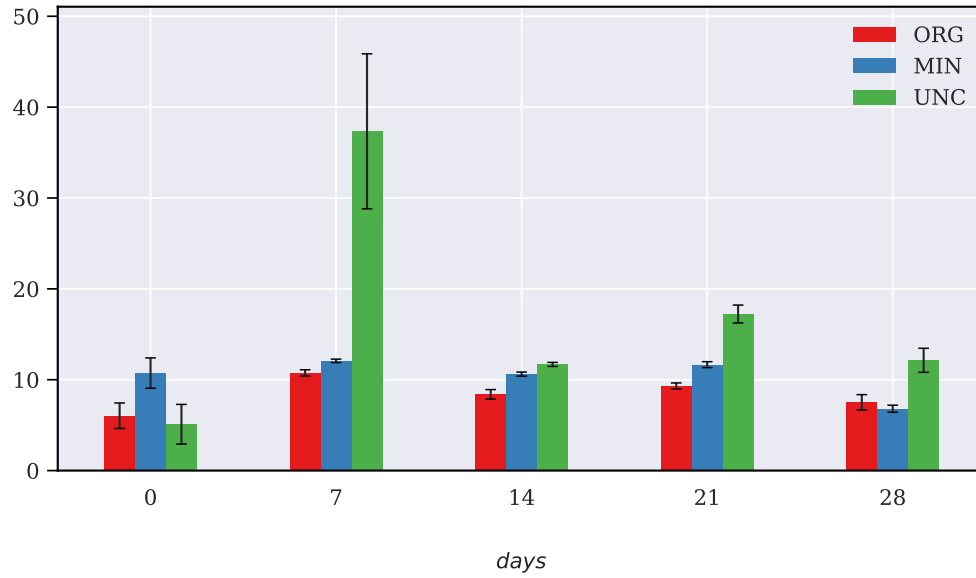


Figure 34: C/N ratio in MRE amended samples

Aggregate stability

The percentage of stable aggregate was increased by more than 20% in the course of the incubation in all three soils (Table 4). For ORG and UNC, ~80% of the stable aggregates formed during the incubation period were formed in the first half of the incubation, while MIN samples presented almost similar increases in percentage of stable aggregates in the second half as in the first half of incubation.

Table 4: Aggregate Stability in MRE treated samples

soil days	MIN	ORG	UNC
0	19.33 ± 0.89	18.90 ± 0.89	10.42 ± 0.81
14	31.87 ± 2.72	36.71 ± 1.69	28.30 ± 2.45
28	45.47 ± 1.92	41.57 ± 1.49	34.04 ± 1.81

Ergosterol-to-microbial biomass (ERG-to-MBC)

The ratio of ERG-to-MBC dropped sharply in the first week of incubation in both ORG and UNC, with a more moderate decrease in MIN, and reached a similar value of roughly 0.05% in all three soils. From then onwards, ERG-to-MBC remained relatively steady, with a slight decrease and then increase in the fertilized soils, during the 2nd and 4th weeks respectively. By the end of 4 weeks of incubation, ERG-to-MBC values were practically identical at ~0.03%, again indicative of the strong effect of MRE treatment compared with the effect of long-term management treatments.

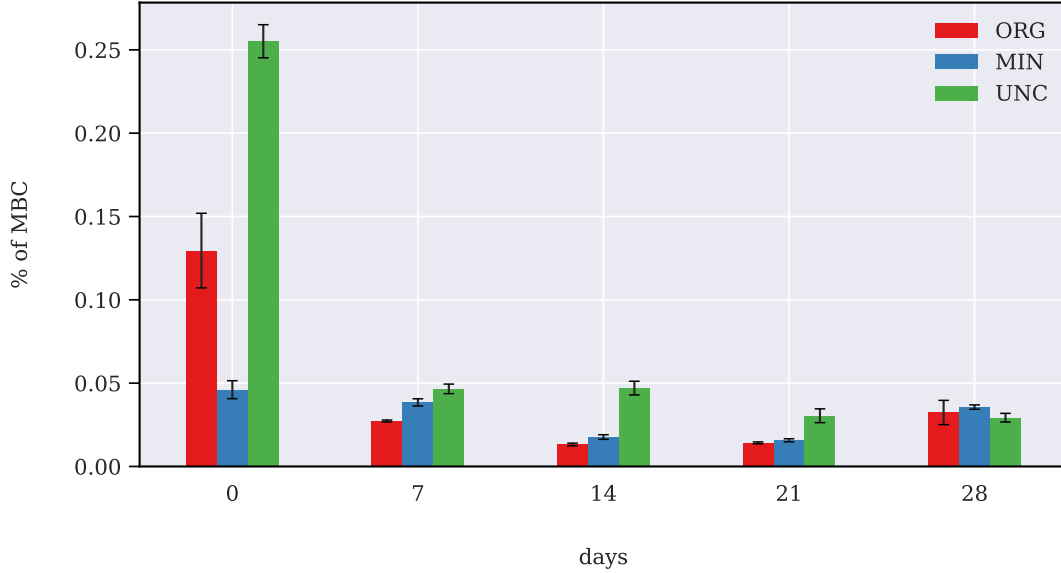


Figure 35: Ergosterol in MRE amended samples

Carbon Use Efficiency

The weekly trend in CUE (Table 5) seemed to differ between the cultivated LTTs and the non-cultivated LTT. Both cultivated LTTs presented a sharp decrease of ~50% in CUE between the 1st and 2nd week with values staying practically the same between the 2nd and 3rd week. by contrast UNC saw an even more pronounced decrease in CUE between the 1st and 2nd week (about 10 fold) while the 3rd week actually saw a sharp increase compared with the 2nd week, with a CUE similar to that observed in the 1st week.

Table 5: weekly Carbon Use Efficiency in MRE treated samples

soil week	MIN	ORG	UNC
1	0.40 ± 0.06	0.46 ± 0.02	0.37 ± 0.04
2	0.23 ± 0.06	0.20 ± 0.03	0.05 ± 0.04
3	0.20 ± 0.07	0.20 ± 0.05	0.42 ± 0.07

Bibliography