Functional Compost

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SUMMARY:

The aim of the research program Functional Compost is to develop and test compost, which have been enriched with chitin, for plant growth promoting properties and to recognise specific mechanisms. Two types of compost were included in the program: source separated biodegradable municipal solid waste compost (DM = 62 %) and garden and park waste compost (DM = 66 %). Chitin was added in trace amounts during the maturity phase, combined with two levels of trace amounts immediately before adding the compost to the growth medium. The research program includes several parallel experiments. In experiment I, compost (20 vol. %) was added to soil (no plants) and incubated at 15 °C for 5 month, under regular determination of microbial respiration and gross and net N mineralization. There was a significant increase in respiration due to chitin enrichment, which could not be explained by the amount of C derived from the chitin, which therefore suggest a priming effect. The N analyses are still being processed in the laboratory, but data are expected to be available at the conference. In experiment II, compost was mixed with sand, put into pots in a climate chamber, and spring barley seeds infected with Fusarium culmorum were sown in the pots. After 3 weeks of growth, the health of the plants was determined, and the chitinase activity in the sand was measured. The health of the plants and the chitinase activity was significantly higher in the treatments receiving municipal waste compared to the treatments receiving garden waste compost. However, there was no clear effect of the chitin enrichment. Additionally, the microbial community structure of the two types of compost, with and without early chitin, was determined by Denaturing Gradient Gel Electrophoresis (DGGE). There was a clear separation between compost types, and with or without early chitin amendment. Experiment III is a regular growth experiment, and is running right now. Compost has been incorporated into soil, put into pots in the greenhouse, and spring barley is grown for 2 month before determination for wet and dry weight and N uptake. Data from experiment III is expected to be available at the conference.

Compost, chitin, soil application

1 Introduction

The main hypothesis of the project is that compost, enriched with chitin, will promote specific microorganisms when applied to soil, and that these microorganisms will have a beneficial effect on the plant production. Such effects has e.g. been proposed by Hoitink & Boehm (1999), who suggested that transcriptional activity of chitin-degrading enzymes of *Trichoderma* spp. is repressed in the presence of the more favoured cellulosic substrates. These antagonistic microorganisms can control pathogenic fungi through enhanced chitinase activity. In addition, it is expected that an enhanced enzyme activity will promote an increased release of nutrients from the soil. First of all, the knowledge produced in this project, will be beneficial for the production of a high-value compost product. Secondly, the knowledge can be the foundation for improvement of other organic soil improvement products. The overall aim of the project is to investigate whether chitin enriched compost applied to soil has growth promoting properties, and if so, to document the mechanisms behind these properties.

2 Materials and method

Two types of compost were used in the project: Compost based on garden/park-waste (D-Gro A) and compost based on source separated municipal solid waste (Biovækst). During maturation 0 or 0.05 % chitin was applied (early chitin). After maturation, additional chitin was applied: 0, 0.05 or 0.25 % (late chitin).Thus in all 12 compost types was produced:

1) D-Gro A: No chitin
2) D-Gro A: 0.05 % early chitin
3) D-Gro A: 0.05 % late chitin
4) D-Gro A: 0.25 % late chitin
5) D-Gro A: 0.05 % early chitin + 0.05 % late chitin
6) D-Gro A: 0.05 % early chitin + 0.25 % late chitin
7) Biovækst: No chitin
8) Biovækst: 0.05 % early chitin
9) Biovækst: 0.05 % late chitin
10) Biovækst: 0.25 % late chitin
11) Biovækst: 0.05 % early chitin + 0.05 % late chitin
12) Biovækst: 0.05 % early chitin + 0.25 % late chitin

These 12 composts were used in the following experiments: antagonist effects, chitin activity and C and N mineralization. In addition, the relation between microbial diversity and chitinase activity in compost 1), 2), 7) and 8) was examined.

2.1 Disease suppression and plant growth promotion

Sand was mixed with the different composts (20% ww). Barley seeds (Prestige)- harvested in 2004 and tested near free for pathogens (< 1%) – was coated with *Fusarium culmorum* in a suspension with 1.5×10^6. cfu per ml. Suspensions were made from washing sphagnum/bran inoculum. Concentration in sphagnum/bran inoculum was 1.6×10^7 per g. Seeds were infected the day before the experiment was started, by shaking 80 g of seeds in 160 ml in 30 minutes following by drying.
The experiment was made by 5 replicate, where each replicate consisted of 6 pots in a series. In each pot 3 seeds were sown. At day 0, 5, 14 and 21, 2 g soil from each replicates was sampled for determination of chitinase activity. At day 21, rhizosphere soil was also sampled for chitinase activity.
After 21 days, the experiment was finalized. Disease severity was determined according to an index from 0 to 4, where 0 is healthy plants and 4 is non-germinated seeds or dead seedlings (Knudsen et al. 1995). Furthermore, the plants were weighted for dry matter determination.

2.2 Chitinase activity

The fluorogenic substrate 4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide was used for the quantification of β-N-acetylglucosaminidase (EC 3.2.1.30) activity, i.e., chitinase activity. The chitinase activity was detected as fluorescence from liberated 4-Methylumbelliferone (4MU) and was measured using a fluorometer with micro-titre plate reader at 377 nm excitation and 446 nm emission. The activity was expressed as nmole 4MU liberated per hour per g (dry weight) of compost. To correct for quenching, standard curves from 4MU were made by standard addition to quenching samples.

2.3 Carbon and nitrogen mineralization

This experiment was run concurrently with the disease suppression and chitinase experiments. While the above mentioned experiments were conducted in sand as a growth medium, this experiment was conducted in a loamy soil. The 12 composts were mixed into the soil (20 % ww) and incubated at 15°C for 114 days. Respiration and net N mineralization was measured ad day 0, 7, 28, 56 and 114, and gross N mineralization-immobilization-turnover (MIT) was determined during the time intervals: day 5-7, day 26-28 and day 112-114. CO_2 was trapped in a 0.5 M NaOH solution, and respiration was determined by back titration of the remaining NaOH. Soil was

extracted with 1 M KCl and filtered. Mineral N in the soil extracts was determined by flow injection, and net N mineralization was determined as the temporal pattern of mineral N content. ^{15}N labelled NH_4^+ was applied to soil at day 5, 26 and 112, and the soil was extracted with KCl after 2 hours and 2 days. Gross N mineralization was determined as the dilution of the ^{15}N abundance NH_4^+ pool, and gross N immobilization was determined as the enrichment of ^{15}N in the organic-N pool (Murphy et al., 2003).

2.4 Microbial diversity and chitinase activity

The microbial diversity in compost 1), 2), 7) and 8) was examined by Denaturing Gradient Gel Electrophoresis (DGGE). DGGE was performed using the D-GENE System (Bio-Rad) DGGE equipment. The relative intensity of a specific band was expressed as the ratio between the intensity of that band and the total intensity of all bands in that lane. Principal component analysis (PCA) was performed using The Unscrambler (Version 8.0, Camo). The relative data of the DNA bands from the bacterial and fungal communities were analysed to detect grouping patterns among the composts.

3 Results and discussion

3.1 Disease suppression and plant growth promotion

The experiment showed that plants growing in sand mixed with garden/park compost were in general more diseased, compared to plants growing in sand with MSW compost (Fig. 1). The plants growing in pure sand were more diseased than the plants growing in sand with compost, no matter what compost type. Earlier experiments have shown that soil texture have an importance for disease suppression of brown rot in barley caused by *Fusarium culmorum*, but this disease suppression was also influenced by biotic factors (Knudsen et al., 1999). Chitin amendment did not support disease suppression statistically (Fig. 1) which indicates that antagonistic microorganisms have not been stimulated by different chitin amendments.
The plant growth were promoted with compost amendment compared to sand without compost (Fig. 2) However, only a small difference between different compost treatments were registered. Best results were obtained with MSW compost amended with the highest amount of chitin. Pre-experiments showed that compost also promoted growth of uninfected barley seedlings. Therefore, it is expected that the plant growth promoting effect in the experiment can be a result - not only of plant disease suppression - but also of enhanced nutritional conditions.

3.2 Chitinase activity

The chitinase activity (Table 1) was generally higher in the MSW compost samples then in the samples from the garden/park compost. Based on the results, it was unclear whether the chitin amendments had effects on the chitinase activity, since there were large deviations between replicates. The two late chitin levels did not show any clear picture during the 21 day experiment, however, there was an indication that the activity was increasing during the experiment for the 0.25 % late amendment to the MSW compost (Fig. 3). There was no clear difference in chitinase activity between bulk soil and rhizosphere soil.

3.3 Carbon and nitrogen mineralization

So far results for net and gross N mineralization are not available. Only results for the respiration are available. The results showed a clear effect of chitin amendment. The respiration was lower in garden/park compost than in MSW compost, and for both composts, the early chitin amendment gave rise to increased respiration (Fig. 4). The respiration from MSW compost with early chitin amendment got an extra bust by additional late amendment (data not shown). The amount of chitin applied was so little, that C mineralization from the chitin could not account for the increased respiration. Hence, the addition of chitin must have caused a priming effect. This could either be increased C mineralization from the soil organic matter, the added compost or soil microbial biomass. Carbon and N mineralization are closely related (Luxhøi et al., 2006), so it is very likely that there also are a

priming effect on the N mineralization, however, this can not be verified before the N samples has been analyzed in the laboratory.

3.4 Microbial diversity and chitinase activity

Results from the experiment with the microbial diversity and chitinase activity in compost 1), 2), 7) and 8) are presented elsewhere (Poulsen et al., submitted). The MSW compost had higher chitinase activity then in the garden/park compost. For both composts, the early chitin amendment resulted in higher chitinase activity compared to composts without early chitin amendment. The DGGE profiles separated the microbial communities from the four composts from each other. This was both with regard to bacteria and fungi. A PCA showed that variation that could be explained from the axis's were PC1: 57%, PC2: 20% for bacteria and PC1: 48%, PC2: 16% for fungi. This indicates that the original microbial communities in the two main composts are different, and that early chitin amendment also will change microbial communities.

4 Conclusion

In conclusion, the two different compost types are of different quality regarding microbial composition, chitinase activity and respiration. The plant assay demonstrated that these effects resulted in different level of disease suppression in the two compost types. However, no general effect on plant growth or disease suppression could be registered reflecting chitin amendments. Based on the marked effect of chitin amendment on respiration, and since there is a well known link between respiration and N mineralization, we suspect a marked effect of chitin amendment on N mineralization, but this has not been evaluated yet. Concurrently we are running a regular growth experiment. It is too early to state any thing about this experiment, but it will be very interesting to see, whether the expected accelerated N mineralization, and the changed microbial community will result in growth promoting properties.

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Figure 1 Disease index after three weeks of growth in sand with and without application of 20 % (ww) garden/park or MSW compost. In addition, to some treatments early or/and late chitin have been amended. Disease severity was determined according to an index from 0 to 4, where 0 is healthy plants and 4 is non-germinated seeds or dead seedlings. Letters denotes treatments that are significantly different (P= 0.05).

Figure 2 Dry matter (g/pot) of harvested barley plants after three weeks in sand with and without application of 20 % (ww) garden/park or MSW compost. In addition, to some treatments early or/and late chitin have been amended. Letters denotes treatments that are significantly different (P= 0.05).

Figure 3 Chitinase activity determined in samples consisting of sand/MSW compost (A) and sand/garden/park compost (B) with ealy chitin amendment. Samples with 0 % late chitin (plain), 0.05% late chitin (chequered) and 0.25% late chitin (hatched).

Figure 4 Cumulated respiration (A) and respiration rate (B) for soil, and soil with incorporation of one of the four compost types. The graphs present mean values ± STDEV of 3 replicates.

Table 1 Chitinase activity determined in sand/compost from disease suppression experiment. Numbers in brackets are one std (n=5). ud=under detection limit.

Table 1.

Treatments	Chitinase activity (nmol 4MU/hour x g DW)				
	Bulk-soil				Rhizospherere soil
Day	0	5	14	21	21
1 sand	5(10)	ud	11(22)	4(13)	6(8)
2 garden/park	11(3)	ud	17(23)	23(16)	Ud
3 MSW	55(25)	21(24)	57(22)	30(21)	52(10)
4 garden/park + early chitin	4(10)	ud	6(30)	20(22)	12(8)
5 MSW+ early chitin	207(86)	46(17)	50(54)	45(50)	77(35)
6 garden/park + 0.05% late	ud	ud	4(32)	23(42)	15(19)
7 MSW + 0.05% late	41(7)	64(27)	68(48)	57(49)	89(52)
8 garden/park + 0.25% late	ud	7(8)	93(47)	78(113)	30(16)
9 MSW + 0.25% late	31(35)	49(22)	110(66)	109(158)	149(147)
10 garden/park + early chitin + 0.05% late	ud	98(218)	49(43)	37(45)	32(25)
11 MSW + early chitin + 0.05% late	189(71)	67(35)	75(28)	53(46)	87(23)
12 garden/park + early chitin + 0.25% late	61(132)	17(28)	66(69)	17(12)	61(71)
13 MSW+ early chitin + 0.25% late	139(18)	75(71)	119(95)	206(58)	78(51)

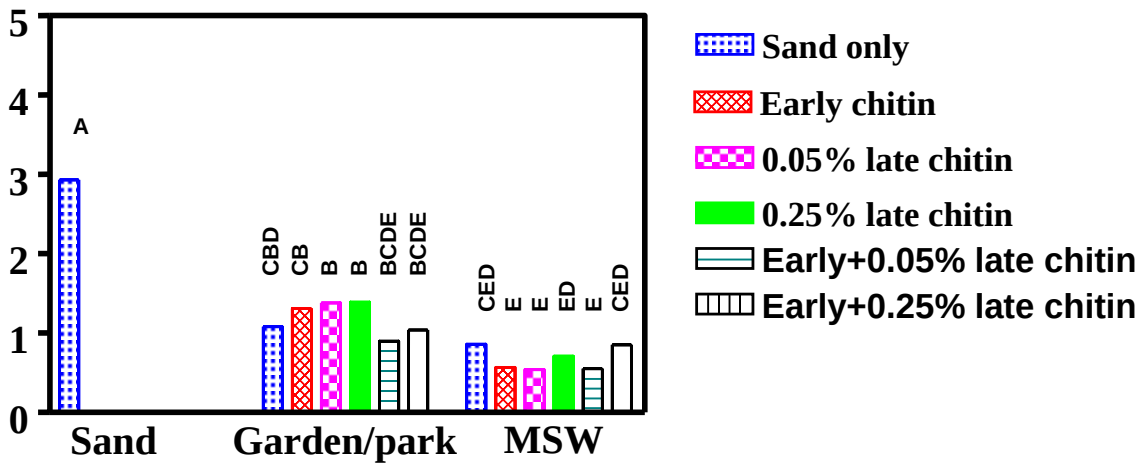
5
4
3
2
1
0
A
CBD
CB
B
B
BCDE
BCDE
CED
E
E
ED
E
CED
Sand
Garden/park
MSW
Sand only
Early chitin
0.05% late chitin
0.25% late chitin
Early+0.05% late chitin
Early+0.25% late chitin

Figure 1

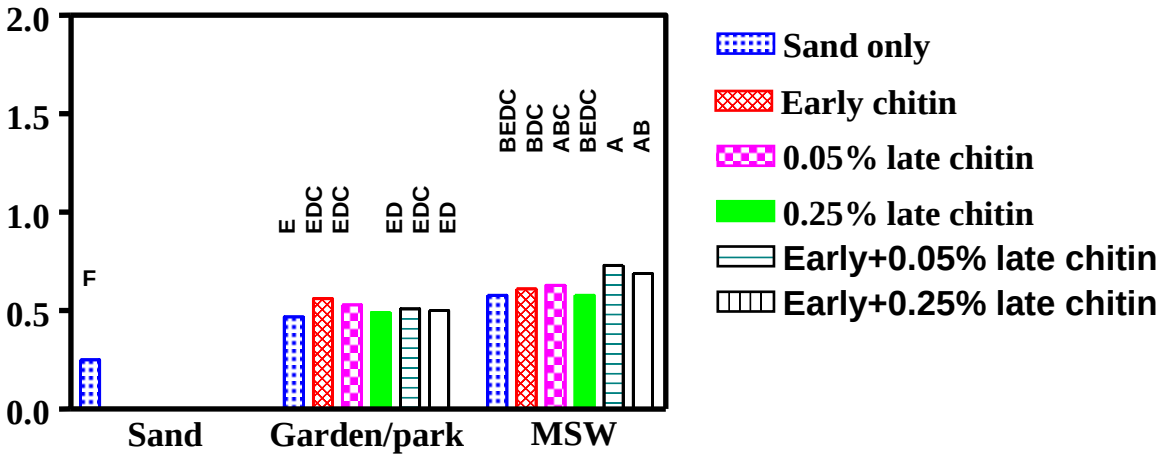
2.0
1.5
1.0
0.5
0.0
F
E
EDC
EDC
ED
EDC
ED
BEDC
BDC
ABC
BEDC
A
AB
Sand
Garden/park
MSW
Sand only
Early chitin
0.05% late chitin
0.25% late chitin
Early+0.05% late chitin
Early+0.25% late chitin

Figure 2

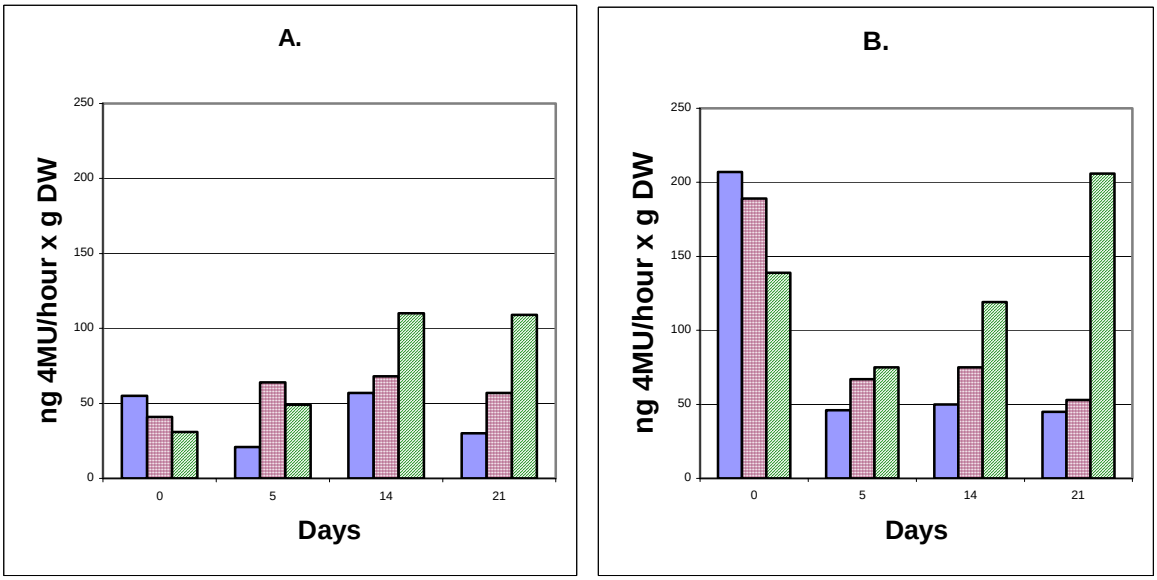
A.
ng 4MU/hour x g DW
250
200
150
100
50
0
0
5
14
21
Days
B.
ng 4MU/hour x g DW
250
200
150
100
50
0
0
5
14
21
Days

Figure 3

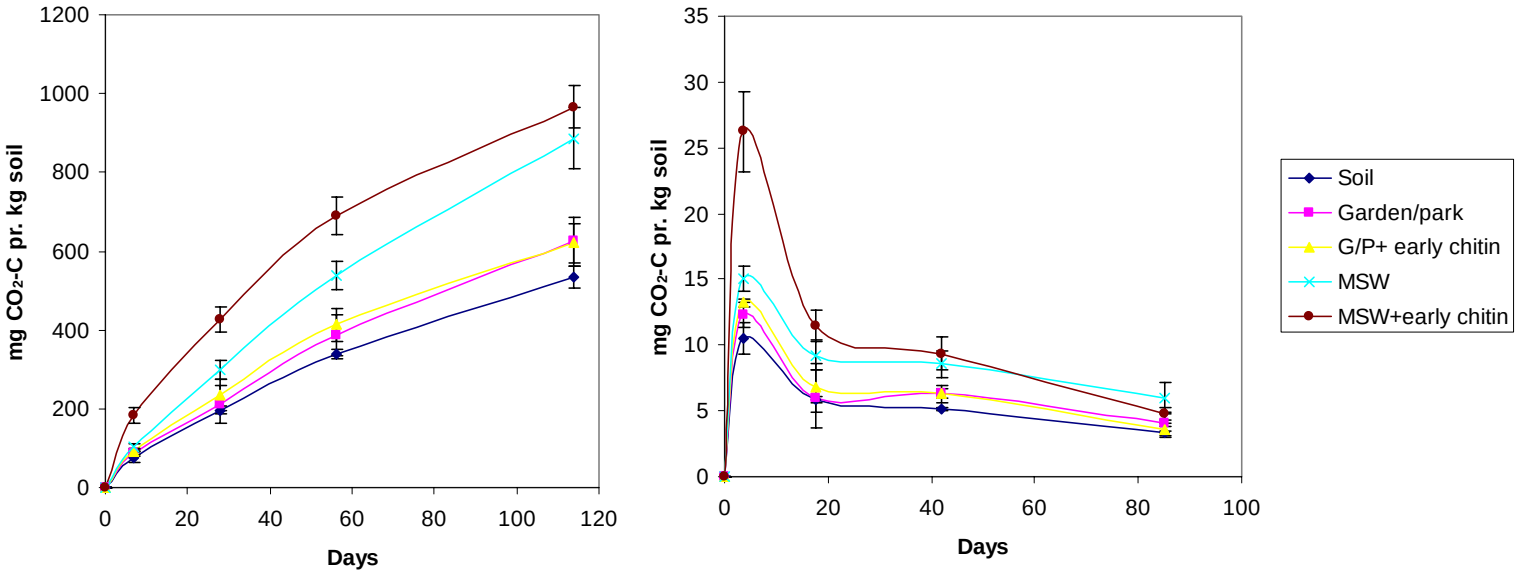
mg CO2-C pr. kg soil
Days
Soil
Garden/park
G/P+ early chitin
MSW
MSW+early chitin

Figure 4