Ph.D. dissertation · **Dorte Bodin Dresbøll**

Optimisation of growing **media for organic** greenhouse production

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Preface

This Ph.D.-dissertation has been submitted to the Royal Veterinary and Agricultural University (RVAU) in partial fulfillment of the requirements of the degree of Doctor of Philosophy. The Ph.D. project has been carried out at the Danish Institute of Agricultural Sciences (DIAS), Department of Horticulture and at the Institute of Agricultural sciences, Plant and Soil Science Laboratory (RVAU). Most of the analyses have been performed using the equipment of the Plant and Soil Science Laboratory apart from for the Scanning Electron Microscopy studies, which mainly were conducted at the University of Aarhus.

The Ph.D. project has been funded partly through a scholarship in the Research School for Organic Agriculture and Food Systems (SOAR) and by the Danish Research Center of Organic Farming (DARCOF) through the project ORCTOM. The overall aim of the ORCTOM project was to develop alternative growing systems for greenhouse vegetables. The effects of growing plants in compost in a closed system or in compost in an intermediate system where roots were allowed to penetrate into the soil were compared to traditional growth in soil. Finally the remaining funding was granted by The Danish Directorate for Food, Fisheries and Agri Business through a project on organically grown potted plants.

The dissertation deals with different aspect of composting plant residues and is based on a general review on optimisation of growing media in organic greenhouse production and four manuscripts (appendixes I, II, III, IV) presenting the main part of the research. Three manuscripts (I, II, IV) have been submitted for publication and the remaining manuscript is in preparation (III).

Paper I describes how management of the composting process can alter the mineralisation pattern. By postponing the addition of some of the nutrient rich material almost twice as much nitrate was available after 8 weeks of composting. Paper III follows up on these studies and determines how the postponed addition affects long-term stability and mineralisation rate in a semi-natural environment. Paper II illustrates visually how different straw materials are being degraded and how the anatomical arrangement affects this degradation. By the use of Scanning Electron Microscopy, structural properties suitable for being used in compost as growing media were clarified.
Finally, paper IV describes the differences in structural properties between three composts based on different straw materials.

Acknowledgements

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I am very thankful to Stig Sandholt Andersen, Jens Barfod and Knud-Erik Pedersen for cutting and mixing – without you no compost was ever made. My appreciation also goes out to Anne Clemmensen. Anne shares my interest in physical structure of substrates and together we shared the joys and worries of the sandbox.

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Contents

Summary

Organic production of greenhouse crops has developed during the last decade. Greenhouse crops in general, have higher nutrient demands than field grown crops and therefore, in order to optimise production it is essential to focus on the growing media and fertilisation. The studies comprised in this dissertation describe the suitability of compost as a growing medium for organic greenhouse crops. The choice and composition of plant material as well as shredding of material and management of composting processes can alter the nutritional and structural quality of compost based on plant residues. This dissertation is based on several studies on the nutritional quality of compost as well as studies of the structural properties of different straw materials and how these are transformed during composting.

Composting is an aerobic process driven by microbial activity. The process is highly dependent on a wide range of different parameters such as temperature, pH, and moisture content. In addition, the nutrient content and structural properties of the material to be composted influence the end product.

The availability of nutrients is important when plant-based compost is used as growing medium. However, mineralised nitrogen is generally only present in small amounts in compost due to immobilisation processes and nitrogen losses during the process. Thus, management of composting processes in order to obtain more mineralised nitrogen could improve the compost as a growing medium. In the first study, nitrogen mineralisation was managed by postponing the addition of part of the nutrient rich material by 3 weeks. One fourth of the nutrient rich material was added at the start, as this was expected to be sufficient to decompose the readily available carbohydrates of the structural straw material, without a large amount being immobilised. The remaining nutrient rich material was added after 3 weeks, when decomposition was expected to be dominated by microorganisms with a less N demanding metabolism degrading more recalcitrant carbohydrates, thus leading to more mineralised and less immobilised N. The results showed that the mineralisation pattern was altered, resulting in almost twice as much of mineralised nitrogen after 8 weeks of composting as compared to the control where all material was added initially. This has proved to be a simple way of influencing the composting processes without altering the type or amount of material used. In the second study, the mineralisation pattern was followed in a long-term experiment revealing that even in nutrient poor compost, postponed addition had a significant effect on the mineralisation rate, although the effect was much delayed compared to the nutrient rich compost. However, the experiments showed that the stability of the compost was not satisfactory.

Structural quality of the compost is also a key factor in the successful use of compost as a growing medium. Decomposition of plant material is not only dependent on chemical composition but on anatomical arrangement of tissues as well. In the third study, the decomposition of three different straw materials; wheat (*Triticum aestivum*), hemp (*Cannabis sativa*), and Mischanthus (*Mischanthus ogiformis*) were examined in order to get a basic

understanding of the extent and rate of decomposition, and most importantly to evaluate the remains of the decomposition process. These changes were examined by the use of Scanning Electron Microscopy, and revealed great differences in the appearance of the straw materials after composting. Due to the post-composting appearance, hemp was suggested to be a suitable structural element to enhance the physical properties of the compost.

The physical properties of the growing medium are important parameters for successful plant growth, as these are related to the ability to adequately store and supply air and water to the plants. The fourth study determined water retention capacity and particle size distribution for compost based on different plant residues. The physical properties of compost are diverse and suboptimal compared to the properties of peat. However, peat is an inert material requiring large nutrient inputs to be suitable as growing medium, and it can be difficult to supply sufficient nutrients from organic sources. Knowledge on structural differences amongst plant species, and how the anatomical arrangement impedes decomposition, improves the ability of producing growing media with suitable structural properties.

In conclusion, these studies contribute to a general and basic understanding of how to manage composting processes and how plant material is decomposed. The mineralisation processes was shown to be altered when postponing part of the nutrient rich material 3 weeks during composting, hence increasing the nutritional quality of the compost when used as growing medium. In addition, the structural studies revealed differences in actual decomposition of different plant materials, not only dependent on lignin content, but more importantly on anatomical arrangement of tissues. This is fundamental knowledge and must be considered when producing a growing medium based on composted plant residues.

Sammendrag

Gennem det seneste årti er økologisk dyrkning af væksthuskulturer blevet mere udbredt. Væksthuskulturer er generelt mere næringskrævende end markafgrøder, og det er derfor vigtigt at fokusere på dyrkningsmedier og gødskning for at optimere væksthusproduktionen. Denne afhandling er baseret på studier af plantebaseret kompost og dets evne som dyrkningsmedium til økologiske væksthuskulturer. Materialevalg, sammensætning af plantematerialer, snitningsgrad af materialet og påvirkning af komposteringsprocesserne er alle faktorer, der bestemmer den ernæringsmæssige kvalitet og de strukturelle egenskaber af plantebaseret kompost. Denne afhandling er baseret på flere studier af den ernæringsmæssige kvalitet af kompost baseret på lettilgængelige planterester, samt studier af de forskellige materialers strukturelle egenskaber og hvordan disse ændres ved kompostering.

Kompostering er en aerob proces, der styres af mikrobiel aktivitet. Processen er afhængig af flere forskellige parametre såsom temperatur, pH og vandindhold. Næringsindholdet og strukturen af det materiale der komposteres har ligeledes indflydelse på slutproduktet.

Når plantebaseret kompost skal anvendes som dyrkningsmedium er tilgængeligheden af næringsstoffer vigtig. Indholdet af mineraliseret kvælstof er dog som oftest lavt i kompost, idet en stor del bliver immobiliseret eller tabt under komposteringsprocessen. For at opnå et højere indhold af tilgængeligt kvælstof, og dermed forbedre komposten som dyrkningsmedium, er det nødvendigt at styre og dermed påvirke komposteringsprocesserne. I det første studium blev kvælstofmineraliseringen i kompost påvirket ved at udskyde tilsætningen af en del af det næringsrige materiale. En andel af det næringsrige materiale blev tilsat fra starten af komposteringsprocessen, da dette forventedes at være tilstrækkeligt til nedbrydning af lettilgængelige kulhydrater i den strukturelle komponent uden en stor del kvælstof blev immobiliseret. Det resterende materiale blev tilsat efter 3 uger hvor omsætningen er domineret af mikroorganismer med en mindre kvælstofkrævende metabolisme. Disse mikroorganismer nedbryder mindre tilgængelige dele, og en mindre mængde kvælstof forventedes derfor at blive immobiliseret. Dette ville hermed resultere i, at en større mængde mineraliseret kvælstof var tilgængeligt. Resultaterne viste at mineraliseringsmønstret blev ændret og at næsten dobbelt så meget kvælstof var mineraliseret efter 8 ugers kompostering sammenlignet med kontrolbehandlingen, hvor alt materialet blev tilsat fra starten. Dette viste sig at være en simpel måde at påvirke komposteringsprocesserne på uden at ændre typen eller mængden af plantemateriale. Det følgende studium fulgte mineraliseringen i et længerevarende forsøg der viste, at selv ved en næringsfattig kompost havde den forskudte tilsætning af det næringsrige materiale en signifikant effekt på mineraliseringsraten. Effekten viste sig dog senere sammenlignet med en næringsrig kompost. De første studier viste desuden at kompost baseret på hvedehalm og kløvergræs hø ikke er stabilt nok som dyrkningsmedium.

De strukturelle egenskaber i kompost er en væsentlig faktor for at brugen som dyrkningsmedium er vellykket. Nedbrydning af plantemateriale er ikke kun afhængig af

kemisk sammensætning men også af den anatomiske opbygning af vævene. Det tredje studium undersøgte nedbrydning af tre strukturmaterialer: hvede (*Triticum aestivum*), hamp (*Cannabis sativa*) og elefantgræs (*Mischanthus ogiformis*). Formålet med dette studium var for at få en grundlæggende forståelse af nedbrydningsraten, og endnu væsentligere for at undersøge, hvad der bliver tilbage efter en nedbrydningsproces. Disse ændringer blev observeret ved hjælp af Scannings Elektron Mikroskopi (SEM) og viste store forskelle i udseendet af materialerne efter kompostering. På grund af hamps udseende efter kompostering blev hamp foreslået at være et passende strukturelt element der øgede de fysiske egenskaber i komposten.

Et dyrkningsmedies fysiske egenskaber er vigtige for en vellykket plantevækst. Det fjerde studium bestemte den vandholdende evne og partikelstørrelsesfordelingen for kompost baseret på forskellige plantematerialer. De fysiske egenskaber ved kompost er meget anderledes og ikke nær så gode som egenskaberne for tørv. Viden om strukturelle forskelle mellem plantearter og hvordan det anatomiske arrangement forhindrer nedbrydning kan forbedre mulighederne for at producere et dyrkningsmedium med passende strukturelle egenskaber.

Disse studier bidrager til en generel og grundlæggende forståelse af hvordan komposteringsprocesserne kan blive påvirket og hvordan plantemateriale bliver nedbrudt. Studierne viste at mineraliseringsprocesserne kan blive påvirket når tilsætningen af en del af det næringsrige materiale under kompostering blev udskudt 3 uger. Hermed blev den næringsmæssige kvalitet af komposten forbedret. De strukturelle studier viste desuden forskelle i den egentlige nedbrydning af forskellige plantematerialer. Nedbrydningen er ikke kun afhængig af lignin indholdet, men i høj grad også af vævenes anatomiske opbygning. Denne viden er essentiel når et dyrkningsmedium bestående af planterester skal produceres.

Objectives

When using compost as an organic growing medium, the nutritional as well as structural quality of the compost is important. In addition, management of the composting processes and knowledge on the effect of this on final compost quality is crucial. Hence, the overall aim of the studies comprised in this dissertation was:

- To increase the knowledge and understanding of N turnover in plant based compost and investigate how this can be managed.

- To examine how plant anatomy affects decomposition in a compost environment.

- To study how the structure of plant-based compost can be influenced in order to provide suitable physical properties for plant growth.

- To combine and utilise this knowledge in developing a compost-based growing medium for organic greenhouse production providing both structural stability and high nutrient availability.

1. Introduction

1.1 Organic greenhouse production

Organic production in Denmark has evolved during the last decades. The number of organic farms has increased from 400 in 1989 to 3500 in 2003 (Danish Plant Directorate, 2003). However, the organic greenhouse production has not followed the same steep increase. The greenhouse vegetable production covers only 6.2 ha (Danish Plant Directorate, 2003) corresponding to less than 5% of total greenhouse vegetable production, and production of ornamentals and herbs are almost non-existing. One of the major obstacles towards organic greenhouse production is controlling the fertilisation in order to obtain sufficient nutrition without risking high leaching losses. When growing plants in closed containers the nutrition is even more important since suitable supplementary organic fertilisers are scarce. In addition, structure and stability of the substrate influence plant growth. Thus, in order to develop organic greenhouse production more focus on nutrition and structure of growing systems is needed.

1.1.1 Vegetables

Most organically grown greenhouse vegetables are grown directly in the soil. In contrast to field grown vegetables, crop rotation is not straightforward in greenhouses. Every crop requires specific greenhouse equipment and rotation would be very costly. Thus, the crops are grown for years in the same soil with the risk of concentrating pathogens in the soil. As chemicals cannot be used in organic production and sterilisation by steaming is questionable due to the effect on the microflora and the high energy consumption, alternative ways of avoiding pathogens in the soil must be considered. Greenhouse vegetables are very nutrient demanding, needing up to 20 times the amount of N of field grown vegetables. Thus, large amounts of organic fertilisers are added to the soil. In addition, the vegetables can be irrigated in excess, presenting the risk of loosing nutrients by leaching. One way of avoiding these potential problems could be by growing the plants in compost in confined beds. After the season the used compost could be added as soil conditioner in the field while fresh compost is added to the beds, in this way the greenhouse production can become an integrated part of an organic cropping system. In addition, leachate could be collected from the beds thus reducing leaching losses. However, many growers see this system as being in conflict with the principles of organic farming, and see the many advantages of growing plants in soil. In addition, establishment of confined beds will be time and labour consuming. Soil can be a more stable and reliable growing medium than compost as the rooting volume is higher and soil functions as a good buffer for water and nutrients. Hence, an intermediate system where plants are grown in confined beds in compost, but where the roots also have the possibility to develop outside the beds, could potentially combine the advantages of the two systems (Sørensen, 2003).

1.1.2 Containerised plants

When organic plants are grown in small containers there are many requirements to the structure, stability and nutrient content of the growing medium. Peat is approved as an organic substrate but increased concern has risen due to the exploitation of these slowly renewable natural resources. Environmentalists throughout Europe have warned against the intensive utilisation of the peat bog reserves, as resources are being depleted and valuable biological habitats destroyed (Carlile, 2004). As large peat reserves exist throughout the world (Lappalainen, 1996) these worries might be overestimated. However, in organic production an increased focus on natural resources and environmental concerns is desirable. Additionally, peat is a very inert substrate and requires large amounts of organic fertilisers during the production. This can cause anaerobic conditions in the containers due to microbial activity and be harmful to plant growth. Thus, alternatives to peat are desirable in organic greenhouse production.

1.2 Compost

1.2.1 Requirements to compost as growing media

When compost is used as a growing medium in organic greenhouse production a number of requirements should be fulfilled. First, the compost structure must be suitable for plant growth. Water retention, air filled porosity and volume weights are important parameters in a growing medium (Gruda and Schnitzler, 2004) and are dependent on particle size and geometry. Hence, the particle size distribution should allow pores of different sizes to be formed with the largest amount in the range 30-300µm. These will retain water, but will not bind it so tightly, that it is unavailable to plants at water pressures relevant for containerised plants (Payne, 1988). Secondly, nutritional quality of an organic substrate is an important parameter. As greenhouse crops have high nutrient demands, and addition of organic fertilisers to plants grown in closed containers can be difficult and time consuming, initial nutrient contents must be high. Organic liquid fertilisers are to be preferred over solid fertilisers, as application is more easily conducted, although the liquid fertilisers can cause a variety of practical problems such as microbial growth in the irrigation system. Another disadvantage of fluid organic fertilisers is that they often are based on wastes from animals, leading to repellent odours. Thus, compost containing an initially large amount of the nutrients is to be preferred. However, a high nutrient content increases the risk of continuous decomposition of the media in the container, leading to anaerobic conditions or unstable compost, loosing a large amount of the media during the plant production time (Jensen et al., 2001; Gruda and Schnitzler, 2004). Additionally, high initial nutrient levels can damage the roots and inhibit plant growth, and will increase the risk of loosing nutrients.

1.2.2. Materials to be composted

In greenhouse vegetable production composted manure is often used as fertiliser. As organic farms and greenhouse production are not always closely located, transport of the manure can be an expensive and laborious process. As growing media for ornamentals, different types of composted waste have also been proposed, but these are not suitable as peat substitutes as they are too compact and often have a high content of nutrient salts and heavy metals (Weinhold and Scharpf, 1997). Alternatively, replacement of the animal manures in favour of easily available composted plant residues could be a more sustainable solution. Nutrient rich plant material such as clover could be grown in the field close to the greenhouse productions, and residues from other field productions such as different straw materials could be used. Compost based on plant residues must contain at least two different plant material types nutrient poor materials such as straw as structural component, and nutrient rich materials such as legumes to supply nutrients for microbial metabolism and subsequent plant nutrition.

Plant growth is dependent on a wide range of nutrients at different amounts. Nutrient release during decomposition is dependent on the nutrient source and varies between nutrients. However, nitrogen is one of the most important nutrients for plant growth and the main focus in this review.

1.3 Composting

Composting is an aerobic process where microorganisms break down organic matter. During this process carbon dioxide, heat and water are produced (Fig.1). It is a dynamic process carried out by a rapid succession of mixed microbial populations. Under optimal conditions, composting proceeds through three phases: 1) the mesophilic phase with moderate temperatures (below 40°C) lasting a few days, 2) the thermophilic phase characterised by high temperatures (40-70°C), which lasts from a few days to several weeks, and finally 3) the cooling and maturation phase lasting from weeks to several months with temperatures from 40°C to ambient temperature (Fig.2). The three phases affects the material and the microbial biomass differently. Stentiford (1996) suggested that temperatures greater than 55°C maximise sanitation, whereas temperatures between 45 and 55°C maximise the biodegradation rate, and microbial diversity is maximised with temperatures between 35-40° C.

The main components of the organic matter are carbohydrates, proteins, lipids and lignin. The capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate (Tuomela et al., 2000). The more complex the substrate, the more extensive and comprehensive is the enzyme system required. Availability of carbon is essential for most microorganisms as carbon serves as an energy source and additionally is incorporated into their cells. Nitrogen as well, is a critical element for microorganisms because it is a component of the nucleic acids and proteins necessary for cell growth and functioning (Tuomela et al., 2000).

Different communities of microorganisms predominate during the various composting phases. Initially composting is dominated by mesophilic microorganisms which break down the readily available carbohydrates such as monosaccharides, starch and lipids (Tuomela et al., 2000). Klamer and Baath (1998) observed a pattern of a fast increase in microbial biomass immediately after the composting process started, followed by a gradual decrease. This could partly be explained by the availability of carbohydrates as follows: Initially fresh substrate was colonised rapidly but as the easily degradable substrates became exhausted a gradual decrease in microbial biomass took place (Klamer and Baath, 1998). Thus, the community structure during this phase is dependent on the chemical composition of the material to be composted as well as physicochemical parameters such as oxygen content, pH and water content. Additionally, the fast increase in temperature during the initial phases of composting is also responsible for the decrease in microbial biomass (Klamer and Baath, 1998).

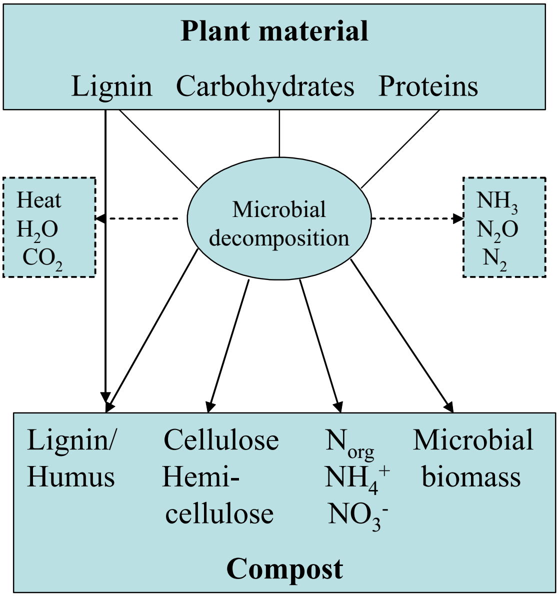

Figure 1. Microbial decomposition - from plant material to compost. Decomposition of plant material during composting is conducted by microorganisms releasing water, CO_2 and heat. Depending on the composting conditions losses of nitrogen can be seen during the process.

Generally, a mixture of bacteria and fungi is seen in this initial mesophilic phase. Due to the activity of these a rise in temperature to thermophilic conditions occurs. As the temperature exceeds 40°C the mesophilic microorganisms become less competitive and are replaced by thermo-tolerant or thermophilic microorganisms. In this thermophilic phase the dominating microorganisms are actinomycetes and species of gram-positive bacteria. Gram negative bacteria and fungi appear to grow only below 50°C (Klamer and Baath, 1998). During the thermophilic phase, the high temperatures accelerate the breakdown of proteins, fats, and complex carbohydrates. Generally, the greatest weight losses are seen during this phase with high temperatures (Bernal et al., 1996).

When the amount of these high-energy compounds becomes in short supply the compost temperature declines and dominance is once again among the mesophilic microorganisms. During the thermophilic phase, fungi, yeasts and streptomyces survives as spores or other dormant structures and re-colonisation proceeds when temperature has declined again. Some microorganisms can survive at the edges of the compost pile as temperature remains lower there; others again are introduced to the compost from the air (Ishii et al., 2000).

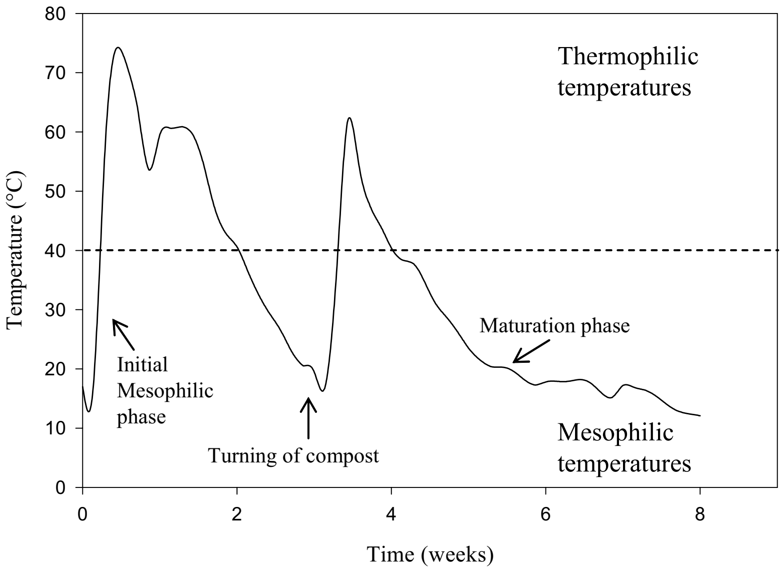

Figure 2. Temperature development during composting of plant residues. Turning the compost can result in a second peak of thermophilic temperatures.

The last phase, the maturation phase, is especially controlled by fungi which are capable of breaking down more recalcitrant compounds. In addition, decomposition of some of the plant material can occur several times throughout the composting period. First it is degraded as plant material and subsequently as decomposition of microbial biomass by other microorganisms. The composting process is considered finished when maturity and stability of the material is reached. Maturity refers to the degree of decomposition of phytotoxic organic compounds produced during the active composting phases and is associated with plant-growth potential, whereas stability refers to the level of microbial activity in the compost (Benito et al., 2003). Maturity and stability are often used as synonyms as both are used to define the degree of decomposition of organic matter during the composting process. Stability is generally determined by O_2 uptake rate, CO_2 production rate or the heat released due to microbial activity. Maturity has been determined by biological methods such as seed germination index, by physical properties such as odour and colour and by chemical parameters such as C/N< 12, NH_4^+/NO_3^- < 0.16 and water soluble carbon < 1.7% (Bernal et al., 1998; Eggen and Vethe, 2001; Benito et al., 2003). However, whether either of these indices can be used is highly dependent upon the material to be composted.

Temperature is a significant factor in determining the relative advantage of some species over another, and is thus the dominant physicochemical parameter controlling microbial activity during composting. However, bacteria proliferating during the composting process also differ with the materials treated. The bacteria appearing during the composting process might have been absent initially or at least representing minor populations in the starting material. Thus, the microbes multiplying in the composting process would be adapted to the composting environment and selected by factors within the composting materials (Ishii and Takii, 2003). The level of composting progression may also be reflected by the amount of dissolved organic C available throughout the composting period. Molecular determination of the microorganisms has revealed differences in microbial species present between the two low temperature phases; the mesophilic and the maturation phase. This suggests that temperature alone does not control the microbial proliferation (Ishii et al., 2000), and the differences in microbial species between the two low-temperature phases was observed as being due to carbon source, pH and the concentration of organic acids and humic substances present (Ishii et al., 2000). Turning the compost during the maturation phase could result in reactivation of the degradation process. This could be explained by incorporation of less decomposed material from the exterior, thereby providing fresh substrate for the microbial biomass (Garcia-Gomez et al., 2003). Additionally, turning the compost affect the microclimate within the compost, altering parameters such as the aeration and moisture content. Finally, mechanical disturbance of the compost could also change the structure of the material and alter microbial communities resulting in increased activity. Thus, turning of the compost could be used as means of producing a homogeneous product and ensure sanitation of all the material.

2. The composting processes

Most plants grown in greenhouses are very nutrient demanding and in organic production the growing medium must contain a part of the nutrients initially. When using composted materials as growing media, knowledge of the nitrogen content and mineralisation processes is an important tool in controlling plant growth.

2.1 N mineralisation-immobilisation-turnover

The degradability of organic material depends on the chemical composition of the material as well as the concentration of N. At the initiation of a composting process most of the N in the material is found incorporated in organic compounds (Fig. 3), principally as part of the structure of proteins and simple peptides (Sánchez-Monedero et al., 2001). The total N concentration can increase during the composting process. This is referred to as a concentration effect and is the consequence of the strong degradation of organic C compounds and loss of C as CO_2 (Sánchez-Monedero et al., 2001). Further, a part of the organic N is mineralised to ammonium (NH_4^+) by the microbial biomass developed in the compost. In general, the highest concentrations of NH_4^+ are found during the first weeks of composting when the degradation is most intense (Sánchez-Monedero et al., 2001). How fast and to what degree the ammonification process arises is dependent on the type and availability of the organic N in the compost (Sánchez-Monedero et al., 2001; Garcia-Gomez et al., 2003).

The NH_4^+ formed during composting undergoes different processes depending on the condition of the material being composted. Under N limitation, NH_4^+ may be immobilised by the microorganisms in the compost, which use it as N source and transform it into organic N. Some of this N will be re-mineralised naturally through turnover of microbial cells. Alternatively, if N is sufficiently available the NH_4^+ may be oxidised to nitrate (NO_3^-) by nitrifying bacteria when the temperature of the mixture is below 40°C and aeration conditions are favourable (Tiquia and Tam, 2000; Volchatova et al., 2002). When NO_3^- is formed, a simultaneous decrease in pH is seen as the nitrification process releases H^+-ions (Eklind and Kirchmann, 2000b; Garcia-Gomez et al., 2003). The intensity of the nitrification process is dependent on the quantity of NH_4^+ available to the nitrifying bacteria. A decrease in NH_4^+ concentration during the initial phase of composting without an increase of NO_3^- can also be seen (Bernal et al., 1996). This can be explained by immobilisation or if the decrease is combined with a decrease in organic N, which could indicate that the produced NH_4^+ was transformed to ammonia (NH_3) and lost through volatilisation (Bernal et al., 1996). This generally occurs when the mixture is at a high temperature and pH is above 7.5, as this favour the ammonium-ammonia equilibrium towards the reduced form (Eklind and Kirchmann, 2000b; Sánchez-Monedero et al., 2001; Witter and Lopez-Real, 1987).

Finally, if anaerobic conditions arise, nitrification will stop, and some organisms will use NO_3^- as an alternative electron acceptor if it is present, a process known as denitrification. When composting is considered to be finished the concentration of NO_3^- should be much higher than that of NH_4^+ ($NH_4^+/NO_3^- < 0.16$) as this in general is considered a measurement of maturity (Bernal et al., 1998). In addition, a high NO_3^- content indicates that aeration has been adequate and the composting processes performed aerobically.

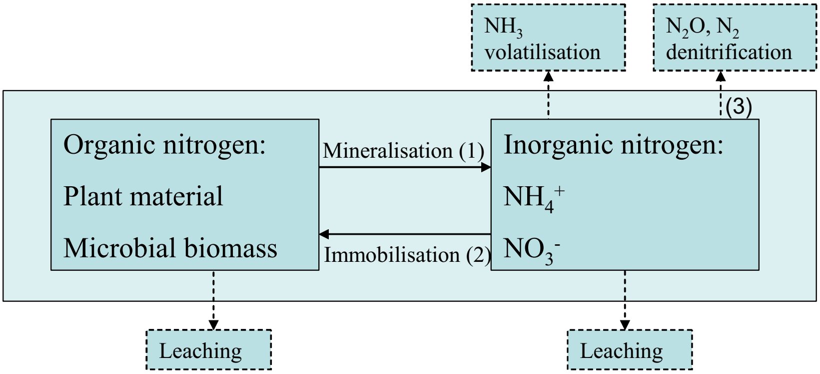

Figure 3. Carbon-driven nitrogen mineralisation-immobilisation turnover in compost. Heterotrophic microorganisms need organic material as a source of combined carbon, as carbon is used as an energy source in respiration and as a component in cell synthesis. To accompany this carbon microorganisms also need nitrogen and various other nutrients. The end product, when decomposing compounds such as proteins, is ammonium which is used by the organisms for cell synthesis. If more nitrogen is present in the organic substrate than is required by the decomposers the surplus will be released to the compost as inorganic nitrogen (1). However, if insufficient nitrogen is present the microorganisms will use inorganic nitrogen from the compost leading to immobilisation (2). Some microorganisms are able to use the oxygen of nitrate as a hydrogen acceptor in the absence of free oxygen leading to denitrification and loss of nitrous oxide and dinitrogen gas (3). Dependent on the conditions in the compost nitrogen can also be lost by leaching if water content is too high or by ammonia volatilisation especially when pH and temperature are high. The broken lines indicate nitrogen losses.

2.1.1. Nitrogen losses

Composting is used as a way of controlling the spread of pathogens, minimising the production of phytotoxic substances, improving storage and handling and reducing unpleasant odours. It is also an effective mean of stabilising organic matter. Thus, composting is considered one of the most suitable ways of disposing unpleasant wastes and can be used to restore and preserve the environment (Tiquia and Tam, 2000). However, composting also reduces the fertiliser value of the material, as large amounts of N, typically up to 50%, can be lost during composting. Losses are partly dependent on the initial C/N ratio and can be so substantial that increases in C/N ratio actually can be observed during the composting process (Tiquia and Tam, 2000). If composting should be considered a sustainable process for organic greenhouse production the N losses must be minimised.
Large amounts of inorganic N is seldom found in mature compost, in general less than 15% of total N (Eklind and Kirchmann, 2000b), as NH_4^+ can be lost as NH_3 and NO_3^- can escape as nitrogen gas via denitrification (Jeong and Kim, 2001). Martins and Dewes (1992) found losses of up to 77% of initial N when composting animal manure. Much research has focused on the N losses during composting and different ways of loss reduction has been suggested. Addition of magnesium and phosphate salts to compost resulted in formation of struvite

crystals ($MgNH_4PO_4 \cdot 6\ H_2O$) binding the NH_4^+ and thus reducing N losses (Jeong and Kim, 2001). Addition of zeolite or clay soil on top of composting piles was shown to reduce the NH_3 volatilisation as well by adsorbing the NH_3, whereas mature compost used as a capturing layer proved to be ineffective (Witter and Lopez-Real, 1988). Lignocellulotic materials have also been found to reduce N losses. Wastes with high content of lignocellulotic compounds lost less than 25% of initial N compared to wastes with low content which lost more than 40% (Sánchez-Monedero et al., 2001). Lignocellulotic materials might reduce the N losses by immobilising the mineralised N. In addition, lignocellulotic materials might improve the structure of the compost reducing the risk of having anaerobic conditions and thus losses due to denitrification.

During composting of animal manure, household waste, and other waste products losses of at least 50% may be observed (Witter and Lopez-Real, 1988; Martins and Dewes, 1992; Eklind and Kirchmann, 2000a). Composting with low total N losses has also been achieved when composting plant residues. Despite conditions permitting ammonia losses, total N losses of 4-29% of initial N content has been observed (Dresbøll and Thorup-Kristensen, submitted A). The low N losses in these experiments could partly be explained by the experimental set-up. The compost was not rotated and aerated as much as compost in reactors (Eklind and Kirchmann, 2000b; Beck-Friis et al., 2001; Beck-Friis et al., 2003) or heaps normally are (Martins and Dewes, 1992; Sommer, 2001). This might reduce the NH_3 volatilisation but could on the other hand also lead to higher risks of losses through denitrification. Additionally, composting time was short compared to other experiments, although this might have a minor effect on the N losses, as the main part is lost during the first weeks of composting (Witter and Lopez-Real, 1987; Sommer, 2001). However, the mineralised N made up less than 5% of total N in the compost after 7½ weeks of composting in the study of Dresbøll and Thorup-Kristensen (submitted A), which might lead to lower losses. When compost is used as an organic growing medium attempts to reduce the N losses must be conducted if compost should be a sustainable alternative.

2.1.2. Measuring N dynamics in compost

When compost parameters are analysed, analyses developed for determining soil properties are generally used. In many cases this provides high-quality measurements, but since compost is a completely different material than soil, problems can arise. In the studies by Dresbøll and Thorup-Kristensen (submitted A), the mineralization patterns were determined by the use of flow-injection-analysis (FIA) using the methods of Keeney and Nelson (1982) with few modifications. The NH_4^+ and NO_3^- content of the compost samples was measured in a KCl extract. Nitrate was determined by reduction to nitrite via a copperised cadmium column, mixed with a diazotizing and coupling reagent and measured spectrophotometrically at 520 nm. NH_4^+ was volatilised to NH_3 by mixing the sample with NaOH, passed through a gas permeable membrane and into an indicator, changing colour due to a pH increment and finally measured spectrophotometrically at 570 nm. The KCl extract became strongly coloured after mixing with the compost, differing between samples from light yellow to dark brown (Dresbøll and Thorup-Kristensen, submitted A). These coloured extracts interfered with the determination of NO_3^- content as the coloured extracts also revealed absorbance at 520 nm resulting in apparent higher NO_3^- contents. As the NH_4^+ was determined by the colour reaction between the volatilised NH_3 and the indicator, the colour of the KCl extract did not interfere with these measurements. No considerations of these problems were found in the literature. To avoid erroneous determination, the samples were cleared with active carbon by shaking for 30 minutes followed by filtration. This cleared the samples and did not interfere with the ammonium measurements. This was tested on cleared and un-cleared samples and no differences were found (Dresbøll, data not shown). However, the clearing process is laborious and time consuming, why alternatives should be considered for future work. One possibility is to run each sample twice with and without reagents to correct for sample colour, although this would prolong the analysis time considerably. Alternatively, other methods of measurements should be used when examining the mineralisation patterns in compost. Determination of NO_3^- by ion chromatography was done by Garcia-Gomez et al. (2002) and also measurements on HPLC might be preferred, as these are independent of sample colour.

When measuring the total N content in compost, choice of method of measurement is also important. Determination of total N by dry combustion can lead to uncertain determination as NH_4^+ contents in compost can be high. When preparing the samples for these analyses the material is oven dried at 80°C (Dresbøll and Thorup-Kristensen, submitted A; Dresbøll and Magid, submitted). The combination of high NH_4^+ content, high pH and high temperature will lead to losses of N as ammonia (Witter and Lopez-Real, 1987). Thus before drying the material, samples were acidified to lower the pH and thereby avoid N losses. Alternatively, total N can be measured by the Kjeldahl method where fresh wet material is used to avoid N losses (Eklind and Kirchmann, 2000b). However, differences in N losses were tested by comparing oven dried material to acidified, oven-dried material and freeze-dried material (Dresbøll, data not shown) and no significant differences were found between the treatments, indicating that N losses might not be a severe problem

2.2 Parameters affecting the composting process

The end product of a composting process is dependent on different parameters. In order to produce a suitable growing medium for containerised plants, it is essential to understand how the composting processes can be influenced, and how the different parameters interact.

2.2.1. C-to-N ratio

C/N ratio is an important variable correlated to mass loss during composting (Eiland et al., 2001a). C/N ratios of approximately 25 have been suggested as optimal for composting (Bernal et al., 1996). If the C/N ratio is above this, a low initial decomposition rate is seen with low respiration rates and low microbial biomass. Most of the nitrogen seems to be immobilised initially when C/N ratio is high, resulting in no net mineralisation (Eiland et al., 2001a). In contrast, if the initial C/N ratio is below 25 this results in a high decomposition rate with high microbial biomass and this will lead to net mineralisation (Eiland et al., 2001a). In general, a negative correlation is found between initial C/N ratio and N loss (Kirchmann and Witter, 1989).

2.2.2. pH

Many waste materials such as household wastes have an initial low pH, often around 5, due to a high content of short chain fatty acids, and pH can decrease further due to release of organic acids during decomposition (Tuomela et al., 2000). During successful and fully developed composting, the pH often rises to 8-9 due to microbial respiration and loss of CO_2. Presence of short chain fatty acids under acidic conditions and their absence when the compost turn alkaline indicate that they are a key factor regulating pH in compost (Beck-Friis et al., 2001). In the transition from mesophilic to thermophilic conditions, a lag phase of stagnation or decline in microbial activity is seen (Beck-Friis et al., 2001; Schloss et al., 2003). This has been noted to coincide with low pH, and Beck-Friis et al. (2001) observed a change in pH from acidic to alkaline conditions when the temperature rose from mesophilic to thermophilic conditions. An explanation for this lag phase could be that microbial respiration is seriously inhibited when the temperature rises above 40°C while the substrate was still acidic (Smårs et al., 2002). In extension to previous studies, Sundberg et al. (2004) showed that the degradation rate of municipal waste differed only slightly with pH values ranging from 5 to 8 as long as the temperature was 36°C. However, if temperature was raised to 46°C, the degradation rate decreased at low pH, but increased if pH was raised to above 6.5. The differences between the degradation rates at 36 and 46°C can probably be explained by the sensitivity of the microbial communities to the combined effects of acidic conditions and temperature. One explanation is that microorganisms can withstand one extreme environmental factor at a time, either high temperature or low pH, but not both simultaneously. Another possibility is the existence of two different microbial communities, a mesophilic acid-tolerant community and a thermophilic community that does not tolerate acids. Fungi in general, are important during the initial mesophilic phase of the decomposition (Klamer and Baath, 1998) and since they are more tolerant towards acid and less tolerant towards temperature than bacteria, this supports the hypothesis that different microbial groups are active at the different temperatures. Additionally, Sundberg et al. (2004) found that the changes in pH during the experiment were different at different temperatures, indicating that fundamentally different metabolic paths were present at the different temperatures.

When composting plant residues, the pH range was found to be much lower than for many waste types (Dresbøll and Thorup-Kristensen, submitted A). The initial pH is generally higher in dried plant material, since the content of fatty acids is much lower than in waste materials. When using wheat straw and clover-grass hay as plant material, the pH initiated at 7 or more, and increased during the first 3-4 weeks. As the amount of fatty acids was low, this increase should be explained by mineralisation of organic N such as proteins during the initial part of the composting process. Mineralisation of organic N is a proton-assimilating process, resulting in the liberation of NH_4^+ and an increase in the pH (Beck-Friis et al., 2003; Tuomela et al., 2000). Towards the end of the composting period, a decrease in pH was seen, which coincided with NO_3^- production as the nitrification process releases protons. Thus, the pH variability is tightly connected to the substrate and the activity of the microbial communities. There is however a general overall pattern during a composting process with increasing pH initially and a decrease when nitrification occurs at the end of the process. Despite the final decrease, pH in compost is in general above 7 or even higher. When growing organic plants in compost, this might be suboptimal as it can be difficult to lower pH in organic systems, and high pH might inhibit the uptake of several micronutrients.

2.2.3. Moisture and oxygen content

Optimum air and moisture contents are important in keeping microbial populations active during composting. The dominant type of metabolism in composting is aerobic respiration rendering oxygen availability very important. Despite the intention of having aerobic decomposition processes, anaerobic conditions will almost inevitable occur in some small zones within the compost pile. Thus, aerobic and anaerobic conditions can co-exist during composting (Beck-Friis et al., 2003). The temperature increase is generated mainly by the aerobic metabolism of microorganisms, and is therefore affected by the availability of O_2. Oxygen in a compost pile is replenished by air flowing into the pile, and hence affected by the air exchange that is dependent of the air-filled space within the heap (Sommer, 2001).

Water is essential to microbial activity and should be present in appropriate amounts throughout the composting cycle. It has even been suggested that moisture content had a greater influence on the microbial activity than the temperature when compost was incubated at different temperatures and moisture contents (Liang et al., 2003). An abundance of water would certainly ease microbial migration and colonisation as well as the diffusion of substrates and metabolic wastes. However, water tends to impede gas exchange as the pores are filled, and a balance should be maintained between the needs for available water and gas exchange. Although water is produced during decomposition airflow generated by the heat convection during composting evaporate significantly more water than is produced and tend to dry the material out (Kulcu and Yaldiz, 2004). The drying of the compost is very unevenly distributed, as the losses occur in vents created within the compost by the airflow. The compost around these vents will have high moisture contents, whereas the areas surrounding them will dry out. If moisture becomes below a critical level, microbial activity will decrease and the microorganisms become dormant. On the other hand, too high a moisture content can cause a lack of aeration creating anaerobic regions within the material (Agnew and Leonard, 2003; Tuomela et al., 2000). Still, the N mineralisation is independent of the moisture content over a wide range, with significant declines only under extreme dry or wet conditions (Amlinger et al., 2003).

Compost structure and water content change dynamically during the composting process. Because moisture affects material and compost properties as well as microbial activity, it has important implications for both the physical and the biological aspects of the composting process (Richard et al., 2002). High moisture contents can affect the strength of the composted material, allowing it to be compressed more easily. Additionally, the decomposition process reduces particle size and increases compost dry bulk density, leading to a reduction in total porosity, which also can lead to production of anaerobic regions (Kulcu and Yaldiz, 2004). The combination of moisture filled pores and compression of the material will also result in an increase in thermal conductivity allowing heat to be conducted more easily away from the compost at thermophilic temperature levels. However, the airflow will be suppressed by the compression, reducing the heat lost this way. In addition, excessively high moisture contents result in free water draining through the compost as leachate (Agnew and Leonard, 2003).

Maintaining an optimum moisture content in dynamic composting systems, where evaporation, metabolic water production and changes in compaction and porosity are all occurring over time, is important and challenging (Richard et al., 2002).
Moisture management requires a balance between two functions, encouraging microbial activity and permitting adequate oxygen supply. Moisture is thus a key variable that affects many aspects of the composting process, from the initial mixture to the mature compost. Generally, a moisture content of 50-60% is considered to be optimal for composting (Agnew and Leonard, 2003). If the moisture content of the material is below 50%, moisture must be applied when initiating the composting process and should be applied whenever necessary during composting. Higher water contents can also result in successful composting as reported by Vallini and Pera (1989), who composted vegetable wastes with an initial moisture content of 82%. Alternatively, high moisture contents could be regulated by adding structural materials of lower water contents to the compost, altering the overall structure and porosity.

2.2.4 Measuring compost process parameters

During the composting process, compost is a very heterogeneous material. The processes are conducted by different microorganisms controlled by competitive advantages of one community over another, and even small changes in the conditions may change the microbial community and thereby the composting processes. Thus, compost can be considered as consisting of several small micro-sites varying in temperature, pH, water content, aeration, nutrient availability and hence microbial communities. During the high activity phase, from the mesophilic to the thermophilic phase, the heat convection generates areas containing more moisture, and can create large vents of several centimetres in the compost piles. These ‘hot spots’ of high activity and heat loss will be significantly different from other sites in the compost and especially from the edges where temperature and water content in general are much lower. Hence, taking representative samples in compost to study compost processes is difficult and in many cases an average will not describe the actual processes adequately.

Compost sampling has been conducted in many different ways, in order to reduce heterogeneity. Eklind and Kirchmann (2000a) pooled 10 subsamples of 250 ml and took samples from the pooled material for further analyses. Sánchez-Monedero et al. (2001) collected one representative sample by mixing six subsamples taken from six sites of the mixture from the whole profile. Before analyzing the samples Sommer (2001) finely chopped samples of 2-l and subsamples was taken from this. In search for a suitable sampling method, sampling was also conducted in different ways by Dresbøll and Thorup-Kristensen, (submitted A) and Dresbøll and Thorup-Kristensen (submitted B). Sample size was kept low in order to reduce disturbance of the composting processes. Initially, 5 samples were collected at 40 cm depth and pooled before subsamples were taken for the various analyses. This method revealed large differences between replicate composting boxes. One explanation for the differences could be that the samples were not mixed thoroughly when pooled, resulting in subsamples, which did not represent the entire box. Thus, in the following experiment sampling was conducted by taking 10 small samples distributed randomly in the boxes for each analysis performed (Dresbøll and Thorup-Kristensen, submitted A). However, this did not reduce the variability between the boxes. It was noted that especially the water content and thereby the dry weight was determined with great variation, affecting many of the other measurements, as these are dependent on dry weight dimensions (Dresbøll and Thorup-Kristensen, submitted A). In the study of Dresbøll and Thorup-Kristensen (submitted B) samples were only collected at initiation, after turning and at the end of the composting period, collecting samples at all three occasions right after mixing the entire composting box thoroughly. This should ensure a more representative estimate of water content of the material and did reduce the variation within the boxes although, interestingly, the variation was still significant between replicate boxes. Thus, this makes the outcome of composting in small scale more uncertain as even small differences can have an immense effect on the outcome, which in larger scale composting experiments might be equalised throughout the pile.

Alternatively, composting in smaller scale can be conducted in compost reactors where process parameters can be controlled leading to more homogeneous compost (Beck-Friis et al., 2001; Smårs et al., 2001; Eiland et al., 2001b). However, the normal dynamics of the composting process will not be expressed and controlling all parameters might affect the microbial succession and provide insight but not necessarily comparable results to large scale composting. Mixing the compost in order to obtain the most homogenous material for analysis was found to be the most effective way of reducing variability. Still, this might restrict sampling frequency when disturbance of the processes by turning the material too much is undesirable (Dresbøll and Thorup-Kristensen, submitted A).

Temperature changes is generally monitored from the middle of the compost material always showing some of the highest temperatures, as temperature in the periphery do not reach the same high temperatures (Dresbøll and Thorup-Kristensen, submitted A; Eiland et al., 2001). Thus, it is important to notice that the temperatures monitored are maximal temperatures and only some of the material will reach such high temperatures.

2.3 Management of the composting process

The initial content of mineralised N in compost used as growing media is important, as availability of nutrients is essential for seedling growth. Management of composting processes in order to control the mineralisation patterns can thus be of great importance.

Control of a composting process and the properties of the end product can be achieved by at least two different strategies. One strategy is to adjust process parameters, such as moisture level, temperature or oxygen content (Beck-Friis et al., 2001; Smårs et al., 2002). Another is to alter the starting conditions by changing the composition or type of material used so that C/N ratio or fibre composition is changed (Eklind and Kirchmann, 2000a; Eklind and Kirchmann, 2000b; Eiland et al., 2001a). A third strategy is to influence the composting process by delaying the addition of part of the material as opposed to including all the material from the start (Dresbøll and Thorup-Kristensen, submitted A; Dresbøll et al., in prep). Thus, without changing material or composting parameters, the properties of the end product can be affected by postponing some of nutrient rich material, probably due to changes in the microbial communities.

The strategy to control the composting processes by postponing the addition of some of the nutrient rich material was based on the hypothesis that efficient compost with high content of available N could be prepared by splitting the addition of the nutrient rich material during the composting process (Dresbøll and Thorup-Kristensen, submitted A). The first addition at the initiation of the composting process should supply sufficient N to support the microbial turnover of the readily available carbohydrates. The remaining nutrient rich material should be added later in the process when the turnover of the wheat straw would already be proceeding. Decomposition of the newly added material would then result in less N immobilisation compared to the compost produced by a single addition at the beginning of the process (Dresbøll and Thorup-Kristensen, submitted A).

In materials with a high C/N ratio, such as wheat straw, nitrogen has often been recognised as a limiting factor for microbial growth and activity during the decomposition of plant material in soil (Recous et al., 1995). However, experiments on the effect of additional N supply to decomposing plant residues in soil showed different results, ranging from increases to decreases of the decomposition rate (Fog, 1988). When adding more N-rich material to compost based on clover-grass and wheat straw, the mineralisation rate was found to increase (Dresbøll and Thorup-Kristensen, submitted A). The effect of added N on decomposition may depend on the plant material, as degradation is influenced by nutrient content and anatomical structure of the material. Parameters such as N source and the time scale of the decomposition process are also influencing the effect of added N. If decomposition is N limited, the effect of added N was shown to have an increasing effect on the decomposition rate although delayed (Dresbøll et al., in prep).

There is a high N demand during the initial stages of decomposition of plant residues in soil when soluble and easily degradable C compounds are mineralised, while the N demand is lower when the more recalcitrant C compounds are decomposed (Recous et al., 1995). Since much C from plant residues such as straw materials is only slowly available to microorganisms, leading to low C use efficiency for growth, a limited amount of N may be required during decomposition, and recycling of N may then be adequate to meet the N requirements (Bremer et al., 1991). Microorganisms, especially fungi, have a considerable capacity to adapt to N deficient conditions. Consequently, an initially large amount of N could lead to gross immobilisation. This could be a result of synthesis of a microbial biomass with a lower C/N ratio such as a change from 'low-N-fungi' to 'high-N-bacteria' or of higher N losses (Bremer et al., 1991).

Dresbøll and Thorup-Kristensen (submitted A) showed that when postponing the addition of some of the nutrient rich material, the mineralisation patterns were altered significantly (Fig. 4). These results supported the hypothesis that a limited amount of N is needed in the initial decomposition of the readily available carbohydrates of the straw material (Bremer et al., 1991). The soluble compounds during the initial phases of decomposition are generally degraded by bacteria, leading to increased incorporation of N into organic compounds, and thus increased immobilisation compared to conditions with low N availability, where fungi with a higher C/N ratio decompose more recalcitrant compounds (Recous et al., 1995; Klamer and Baath, 1998). The fungal/bacteria index increases during the decomposition of material with a high initial C/N ratio in soil. The same phenomenon occurs during the initial phases of composting (Eiland et al., 2001a) confirming the fungal dominance in degrading recalcitrant compounds. When the additional N was added in the study by Dresbøll and Thorup-Kristensen (submitted A), the readily available carbohydrates were presumably already degraded, and less N demanding fungi dominated the decomposition. Thus, when the N was mineralised from the supplemental clover-grass hay it was not re-immobilised by the microbial population to the same degree as when all clover-grass hay was added initially. Therefore the delayed addition of clover-grass hay resulted in a higher total release of inorganic N during the experimental period (Dresbøll and Thorup-Kristensen, submitted A).

The compost from the experiments by Dresbøll and Thorup-Kristensen (submitted A) was used as a growing medium for greenhouse grown lettuce. This revealed that the initial N content of the compost with the postponed addition was too high for lettuce requirements and inhibited root growth. Thus, in the following composting experiment conducted, the initial C/N ratio was increased based on calculations of an expected more balanced nutrient release. Conversely, the changes resulted in a too low initial N input leading to inhibition of the mineralisation processes and almost no net mineralisation was observed during the composting period.

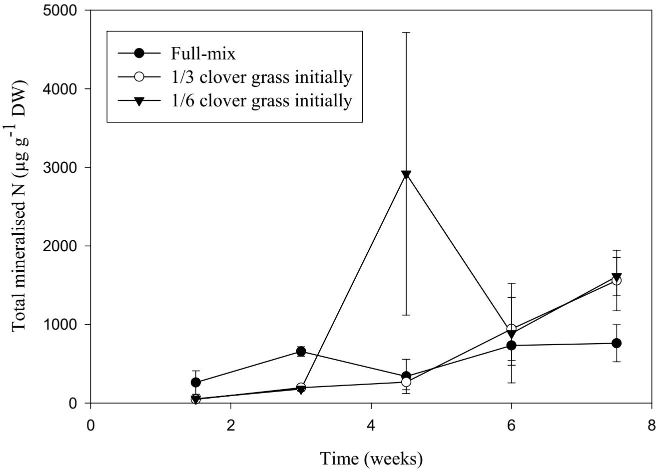

Figure 4. Total mineralised nitrogen in plant based compost with all material initially (full-mix), 1/3 of the nutrient rich matrial initially and 1/6 of the nutrient rich material initially. The remaining nutrient rich material was added after 3 weeks. (Dresbøll and Thorup-Kristensen, submitted A).

Hence, no effect of the postponed addition was observed in this experiment (Dresbøll and Thorup-Kristensen, submitted A). The compost was subsequently used in a leaching tube experiment to follow the further mineralisation pattern for six month (Dresbøll et al., in prep). Regardless of the lack of net mineralisation during the composting time, a significant higher mineralisation was seen in the treatment with postponed addition after 12 weeks of incubation in the leaching tubes (Dresbøll et al., in prep). In conclusion, postponing the addition of some of the material, lead to differences in the mineralisation pattern, independent of the initial nitrogen input. This was probably due to changes in the microbial community structure during composting (Dresbøll and Thorup-Kristensen, submitted A).

3. Compost structure

The structure of plant based compost is dependent on the material, the initial particle size and the degree of shredding. Thus, the structure can be adjusted from the start and knowledge on the extent and rate of decomposition in different plant types can be used to successfully produce a plant based growing media. In general, many decomposition studies focus on what is degraded. However, when using composted plant materials as growing medium the quality of the parts remaining after degradation are equally important.

3.1 Degradation of plant tissue

Degradation of plant material in soil has often been compared to the vast amount of research that has been conducted on the degradation of plant material in the rumen of ruminants (Wilson and Hatfield, 1997; Chesson, 1997). Parallels can be drawn between rumen digestion and soil degradation, as both are microbial processes involving a common substrate and require enzymes with the same specificity to achieve breakdown. Both processes are subject to the same limitations on the rate and extent of degradation (Chesson, 1997). However, there are a number of important aspects where the two systems differ. First, the retention time for plant cells in the rumen rarely exceeds 96 hours, whereas degradation to the same degree in soil will take up to one year. The short time-scale in the rumen does not allow much microbial succession and plant material is simultaneously colonised by fungi and bacteria. In contrast, degradation in soil is dependent on changing microbial communities (Chesson, 1997). Secondly, there is a controlled and near constant environment in the rumen with no nutrient limitation, and anaerobic, aqueous conditions. Finally, the mastication of the plant materials, conducted twice by the ruminants, increases the points of accessibility of the microflora.

The composting process could be perceived as an intermediate system between degradation in soil and degradation in ruminants. Microbial succession and aerobic requirements are similar to degradation in soil whereas retention time, dependent on material and composting system, might be somewhere in between the short retention time in the rumen and the slow degradation in soil. Composting, however, differs from both systems in one important parameter, and that is the high temperature at which part of the degradation occurs. In addition, composting is distinct in that an extremely wide range of microorganisms, comprising mesophilic and thermophilic bacteria, fungi and actinomycetes are present (Atkey and Wood, 1983). In all three systems it can be argued that plant-derived factors determine the maximum rate of degradation, and despite the differences, information on the structural changes during degradation in one system can create a more profound understanding in all three systems.

3.2 Differences in degradability of plant species

3.2.1 Differences in anatomy

Plant species differ in their anatomical arrangement. Roughly, differences in stem anatomy between monocots and dicots can be distinguished by the distribution of the vascular bundles (Mauseth, 1988). However, the distribution of the ground tissues also varies between species of both monocots and dicots and the monocots tend to have more cells with lignified cell walls outside the vascular tissue than do dicots. Besides being dependent on the chemical composition of the material which differs among plant materials, decomposition of plant tissue is dependent on the anatomical arrangement of the tissues. This is because the plant anatomy determines the accessibility for the microorganisms. The cuticle impedes access to underlying tissues and microorganisms can only penetrate through the stomata and lenticels or where the cuticle has been damaged (Chesson, 1997). Initial mastication by animals or shredding of material before composting, increases the microbial accessibility and thereby decomposition rate, whereas decomposition in soil often proceeds at a much slower rate as fallen plant litter is far more intact and microbial attacks thus impeded (Chesson, 1997).

Large differences in degradability of different cell types have been observed to rely on the chemical composition of cell walls. Nonlignified primary cell types such as mesophyll or phloem cells are clearly degraded in preference to the lignified xylem and sclerenchyma (Chesson, 1997; Jung and Engels, 2001). Xylem fibres and primary and secondary xylem vessels appears to be very recalcitrant towards degradation, although these tissues can exhibit differential patterns of degradation depending on the distribution of lignin in their wall structures (Jung and Engels, 2001). Thin walled cells like parenchyma and epidermis occupy an intermediate position between the former. Epidermal cells are often highly degradable in isolation but little attacked *in situ* because they are sandwiched between the inert cuticle and resistant lignified sclerenchyma (Chesson, 1997).

In some cell types, wall material is laid down over the primary wall after cell expansion has stopped. This by definition is secondary wall formation (Harris, 1990) and with only few exceptions, secondary thickening is accompanied by lignification. Unlike sclerenchyma fibers, the secondary wall in the tracheary elements of the xylem is often not laid down uniformly over the primary wall. The protoxylem, which is the first formed tracheary elements and differentiates in organs that are elongating, have lignified secondary wall thickenings laid down on the primary wall in the form of rings and helices (Harris, 1990).

Differences between cell types in presence and form of secondary walls, as well as degree of lignification affect the degradability of the cells. In addition, the arrangement of these cells is a key factor in regulation of the microbial breakdown. Monocotyledonous and dicotyledonous plants differ significantly in the arrangement of cells (Fig.5) and the effect of the arrangement has been examined extensively using grasses and legumes as test plants, as both are used as ruminant feed. Grasses have vascular bundles distributed throughout the parenchyma of stem cross-sections, and lignified cells are not exclusively found in connection with the vascular bundles but are spread across many types of tissues. As a result, degradation of cell walls in most tissues is affected to some extent (Wilson and Hatfield, 1997).

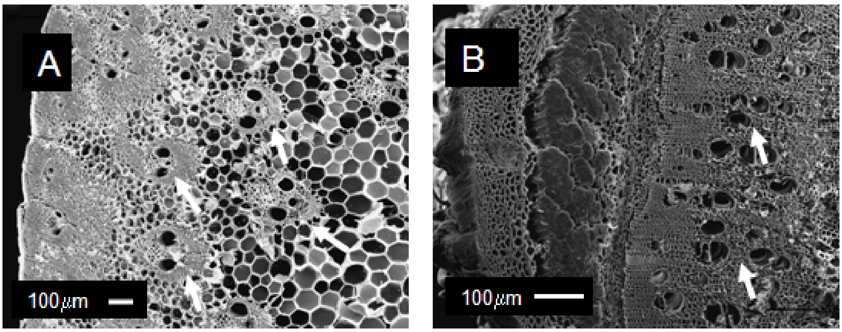

Figure 5. Stem cross-sections of A) Michanthus straw, arrows show the vascular bundles distributed throughout the stem. B) Hemp straw, arrows indicated the solid ring of lignified xylem tissue.

During the maturation of legume stems, isolated vascular bundles soon become unified into a complete lignified xylary ring around the stem that expands through cambial activity (Wilson, 1991; Wilson and Hatfield, 1997). Thus legume stems, from a wall degradability viewpoint, are essentially composed of two populations of cells. The highly lignified xylem ring that appears to be difficult to decompose even though the cells do not have limited microbial access, and the remainder that appears to be easily degraded with no anatomical restrictions to wall degradation because few cortical and central pith cells appear to be lignified, and the cells have a weak structure with a degradable middle lamella-primary wall (Wilson and Hatfield, 1997). The anatomy of many dicots is comparable to that of the legumes and a similar anatomical arrangement was observed in hemp straw (Dresbøll and Magid, submitted). During incubation of clover and grass in soil, these anatomical differences were reflected in the degradability of the material. The clover had an initial high mineralisation rate but the rate decreased after 30-40 days. In contrast, the grass had an initial lower mineralisation rate but the rate did not decrease as fast as for the clover (de Neergaard et al., 2001).

In maturing grass stems, anatomical limitations to wall degradation appear to be of major importance. Secondary walls of all major cell types progressively thicken as the stem matures, and the development of a recalcitrant middle lamella primary wall creates a structurally strong tissue composed of large blocks of cells with thick secondary walls. Microbial degradation is confined to the narrow lumen surface of only those cells with an open end (Wilson and Hatfield, 1997). When microbes have ready access to the surface of cell walls as when digesting thin slices (<100 μm) of material in rumen fluid or when the anatomical structure of lignified tissues is destroyed by isolating and grinding specific cells, then degradation of most of the secondary wall is not prevented by lignification (Wilson and Mertens, 1995). The situation is different for legumes: first, their lignin content is higher than that of grasses at comparable levels of dry matter degradability, and second, the lignin is confined to xylem and tracheary cells only and not spread across many types of tissues as in grasses. This means that in the lignified tissue of legumes, the lignin concentration per unit cell wall will be very high and appears to prevent degradation of secondary walls even when thin sections allow easy microbial access to the walls. Thus, compositional differences between legume lignin and grass lignin may not be as significant as differences in its localisation or concentration in specific wall types, when analysing the contrast in degradation kinetics between legume and grass stems. The poor degradation of xylem elements in the legume stem is almost certainly the effect of their extremely high lignin concentration (Wilson and Mertens, 1995).

The anatomical architecture of the thick-walled lignified fibres of grasses may be as significant a limitation to digestion of secondary cell walls as is the chemical structure (Wilson and Mertens, 1995). This does not mean that lignification of walls and chemical bonding of lignin and phenolics to polysaccharides do not add a further limitation to the rate and extent of wall degradation. Wilson and Mertens (1995) suggested, however, that this effect is largely expressed in the primary wall middle lamella region, where lignin is at a much higher concentration than in the secondary wall. Such an anatomical arrangement influences the accessibility for the microorganisms, as was shown in the degradation of the Mischanthus straw (Dresbøll and Magid, submitted). Degradation of the exposed surfaces of the straw pieces was observed after three weeks of composting whereas a freshly cut surface in the middle of a straw piece did not show degradation until after eight weeks (Fig.6).

Differences were also observed between the dicotyledonous hemp and the monocotyledonous Mischanthus and wheat straw (Dresbøll and Magid, submitted). Although the easily degradable tissues in the hemp straw already had been degraded during the retting process, where the hemp straw is left in the field to initiate decomposition in order to release the bast fibres from the stem, it was obvious that the lignified secondary walls of the xylem were not degraded during the 8 weeks of composting (Dresbøll and Magid, submitted).

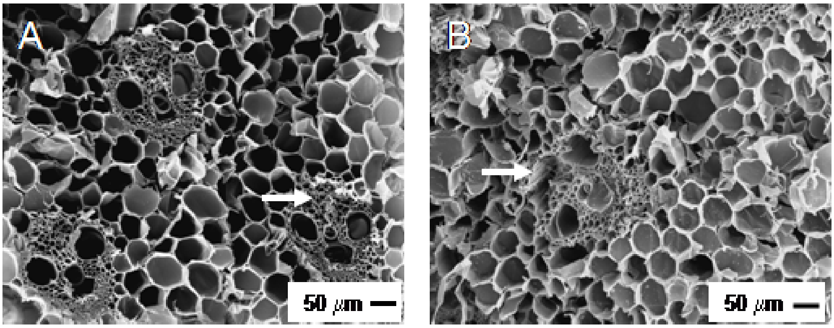

Figure 6. Mischanthus straw after 3 weeks of composting. A) Freshly cut surface in the middle of the straw piece. No visible signs of degradation were observed. B) Exposed surface showing signs of degradation of the phloem (indicated by arrow). After Dresbøll and Magid (submitted).

3.2.2. Degradation of lignin

Anatomical arrangement of the cells within the various tissues is very important in determining the extent and rate of degradation. Nonetheless, a broad inverse correlation exists between cell wall degradability and lignin content. The variation found within any one group of closely related plants also suggests that the nature of the lignin-carbohydrate association can be as important as the total amount of lignin present. Lignin is one of the most recalcitrant compounds in nature and acts to protect the structural polysaccharide to which it is covalently linked. This is consistent with its biological functions, which are to protect structural polysaccharides and ensure rigidity of vascular plants (Hammel, 1997). Lignin can limit cellulose and hemicellulose degradation by impeding enzymatic access to polysaccharides, acting as a physical or chemical barrier (Agosin et al., 1985). The most probable mechanism is a steric hindrance but the hydrophobicity of the complex also moderate hydrolysis. Thus lignin-carbohydrate-complexes (LCC) exposed at the surface of the cell wall are almost resistant to microbial attack, and are either not degraded or are degraded at a rate substantially lower than any surrounding polysaccharide free from any association with lignin (Fig.7). There is a tendency for most LCC to be undermined by the breakdown of surrounding polysaccharides and released from the wall with time, although some LCC accumulate at the wall surface at the expense of non-associated polysaccharides. This changes the surface layer significantly, resulting in slowing the further degradation. The rate at which this inert LCC surface develops is dependent on the initial concentration of lignin in the wall (Chesson, 1997).

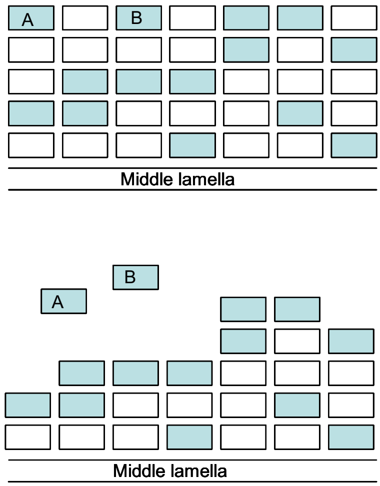

Figure 7. A model of the decomposition of a lignified cell wall seen in cross-section. Microbial attack occurs from the luminal side of the wall. The blue boxes represents regions of polysaccharides closely associated with lignin, whereas open boxes represent free polysaccharides. At the initiation of decomposition the surface (top layer) consists of both lignified and non-lignified polysaccharides. As decomposition proceeds the free polysaccharides are preferentially decomposed leaving a more extensively lignified surface which impedes further decomposition. Some lignin-carbohydrate complex (A and B) is undermined by the degradation of the surrounding polysaccharides and released (Redrawn from Chesson, 1997).

Substrate quality is defined by chemical composition of the decomposing material and has often been considered a critical factor in determining the rate of decay. Nitrogen as well as lignin content of plant material is important in controlling the rate of decomposition. Lignin is an interfering factor in the enzymatic degradation of cellulose and other carbohydrates as well as proteins. High initial levels of lignin may thus slow decomposition rates (Melillo et al., 1982). However, spatial distribution of the lignified cells is a key factor regulating decomposition, as mature lignified secondary walls can be degraded when anatomical factors, i.e. tissue organisation, have been eliminated (Wilson and Hatfield, 1997).

Lignin decomposition is conducted by different groups of microorganisms, depending on the physiochemical conditions of the media. The organisms primarily responsible for lignocellulose degradation are aerobic filamentous fungi (Tuomela et al., 2000). The most rapid degraders in this group are basidiomycetes, especially white-rot fungi. The ability to degrade lignocellulose efficiently is thought to be associated with a mycelial growth habit which allows the fungi to transport scarce nutrients, e.g. nitrogen into the nutrient-poor lignocellulosic substrate that constitutes its carbon source (Hammel, 1997). However, the white-rot fungi do not survive the thermophilic phase of composting, and thus cannot play any significant role in lignin degradation in this environment. Other microorganisms in compost, mainly thermophilic microfungi, are probably the most important lignin degraders. The mineralisation of lignin by compost microorganisms is probably of the same order of magnitude as of a mixed population of soil microorganisms. Lignin degradation in composts is regulated by temperature, the original lignin content and the thickness of the material (Tuomela et al., 2000). Since lignin is degraded by the aerobic fungi of the white-rot and brown-rot species as well as by bacteria to a much smaller extent, the degradation of lignin in aerobic environments is higher compared to anaerobic environments (Komilis and Ham, 2004). Deuteromycetes and other microfungi, which – in contrast to basidiomycetes – are always present in compost, may be involved in the conversion of lignin in compost environments. Ligninolytic peroxidases are considered the most important enzymes in the lignin degradation. The laccases of deuteromycetous fungi seem to be the key enzymes responsible for the low but steady mineralisation of lignin (Kluczek-Turpeinen et al., 2003). In some cases bacteria was also seen to be attached to lignified cell walls (Davis et al., 1992).

In a leaching tube experiment, lignin degradation in composted plant residues was rarely seen (Dresbøll et al., in prep). After almost 8 month of degradation, a small decline in the total lignin mass was observed. During the actual composting process of 8-10 weeks, lignin was not degraded. On scanning electron micrographs it was obvious how thin-walled non-lignified cells were degraded, whereas the lignified cells did not show any degradation (Dresbøll and Magid, submitted). As the hemp straw practically only consisted of the xylem core, the degradation of the non-lignified cells could not be observed. However, the degradation of primary cell walls in the xylem tracheary tissue was observed, revealing the helical secondary reinforcements (Fig.8).
When producing compost of plant residues to be used as a growing medium, knowledge of plant anatomy is of immense importance, as the remaining tissues after degradation determines the overall physical properties of the growing medium.

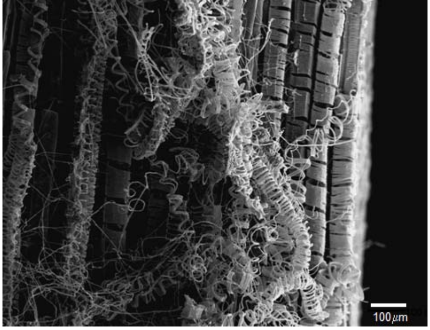

Figure 8. Helical secondary reinforcements of hemp (Cannabis sativa) after 8 weeks of composting.

3.3 Physical properties

The physical properties of compost are key factors in determining its quality as a growing medium. Physical properties are of great concern, because in contrast to the nutritional properties, they cannot easily be changed when inadequate. In horticultural greenhouse systems water is often applied in a short period of time such as in ebb-flood systems or locally as when using drip irrigation. Thus, horticultural media must be able to absorb water fast and be able to distribute the water in the medium. The physical quality of a substrate is related essentially to its ability to adequately store and supply air and water to plants grown in closed containers. The storage and supply of air and water are controlled by pore size abundance, tortuosity and continuity (Allaire et al., 1996). In general, particle size, water holding capacity and air-filled porosity is used as measures of the physical properties of growing media, and an ideal substrate must have a total pore space of more than 85% (Gruda and Schnitzler, 2004). In compost based on plant materials pore size was found to be above 90% (Dresbøll and Thorup-Kristensen, submitted B).

Gas exchange properties are important as well. The air-filled porosity does not provide sufficient information about movement and availability of gasses, and this is regulated not only by the particle size but also by the geometries and pore space continuity (Nkongolo and Caron, 1999), why visualisation of the particles could enhance the understanding of how to produce a suitable growing medium (Dresbøll and Thorup-Kristensen, submitted B). Gas diffusivity or oxygen diffusion rate in growing media is thus thought to be a much better index of aeration than air content or air-filled porosity (Allaire et al., 1996; Caron and Nkongolo, 1999). Similarly, the water holding capacity reveal how much water the media can hold and to some extent the drainage capabilities, but does not bring sufficient information on how easily the media absorbs water. During experiments where the size of bark particles in a growing medium was increased, Nkongolo and Caron (1999) found that the air-filled porosity of the media remained the same while pore tortuosity increased and gas relative diffusivity decreased. It was concluded that when assessing the physical suitability of media for plant growth, the evaluation should not be limited to measurements of storage-related physical properties like air-filled porosity, bulk density and water-holding capacity alone, but should also include gas exchange characteristics such as pore tortuosity and gas relative diffusivity (Nkongolo and Caron, 1999).

The fraction of particles in the size range 0.1-0.5mm is responsible for the plant-available water holding capacity of the media. These particles are expected to produce pores in the range of 30-300μm between them, being optimal for water retention in growing media (Payne, 1988). In green waste compost an even spread between 0.1 and 5 mm was seen in the particle size distribution (Spiers and Fietje, 2000). The largest proportion of particles was also found in this range when examining plant-based composts (Dresbøll and Thorup-Kristensen, submitted B). The finer the material, the higher is the water holding capacity per unit volume;

however a reduction of the particle size leads to a decrease of air capacity and might also decrease the availability of water (Gruda and Schnitzler, 2004).

3.4. Measuring compost structure

Compost structural properties were quantified by measurements of particle size distribution and water retention capacity (Dresbøll and Thorup-Kristensen, submitted B). As these were the initial tentative comparisons between composts based on different structural material, these parameters were expected to be sufficient. Particle size is generally determined by sieve fractionation (Agnew and Leonard, 2003), but this has been found to underestimate the fractions of small particles. Thus, in order to determine small fractions as well, particle size was determined by software analysis of a scanned picture of 1 g compost (Clemmensen, pers.comm.). This method can be criticised by the small sample size which to some extent can be overcome by a high number of replicates. Nonetheless, it has proved to be an illustrative method (Dresbøll and Thorup-Kristensen, submitted B). Beside the quantitative measurement it also provided a qualitative knowledge of the surface appearance and the geometry of the particles visualised at the scanned pictures (Fig.9) (Dresbøll and Thorup-Kristensen, submitted B).

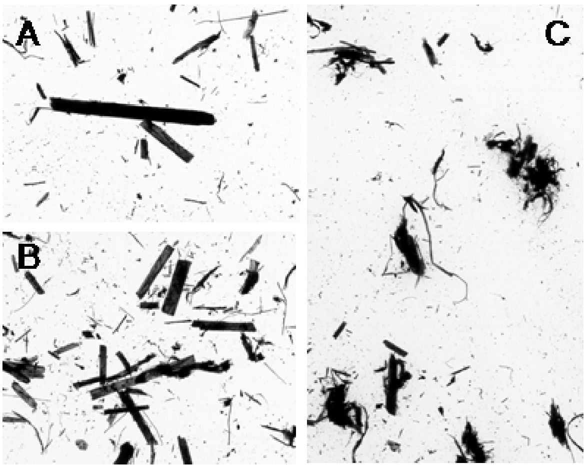

Figure 9. Particle geometry of A) Mischanthus compost based on wheat straw, Mischanthus straw and clover-grass. B) Wheat compost based on wheat straw and clover-grass. C) Hemp compost based on wheat straw, hemp straw and clover-grass (Dresbøll and Thorup-Kristensen, submitted B).

Water retention is generally measured on a sand box keeping compost samples in metal rings and varying the degree of suction (PrEN 13041, 1999; EN 13040, 2000). Suctions can be varied in the range potted plants can experience, although when using compost as a growing medium only part of the compost will probably experience the zero suction where the compost is water filled. Plants will typically be watered by the "ebb and flood"-method and the capillary forces in compost will probably not be sufficient to pull water to the upper layer of the container.

Scanning electron microscopy has been used in some studies following the decomposition during composting (Atkey and Wood, 1983; Davis et al., 1992; Lyons et al., 2000). Examining decomposition of plant material by the use of SEM makes it difficult to quantify the degradation. However, information from the qualitative results should not be underestimated. The visualisation of the actual decay provide valuable insight into how the tissues are degraded, the extent and rate of decomposition, and the spatial distribution of microorganisms (Dresbøll and Magid, submitted). Most important, the SEM studies show the post-composting appearance of the plant material, implying the capability as structural element in compost based growing media. However, when interpreting the scanning electron micrographs it is important to distinguish between signs of decomposition and artefacts caused by the cutting and drying procedures when preparing the specimens for examination.

3.5 Choice of material – anatomical and economical considerations

Plant material to be used as growing medium must be chosen carefully in order to obtain sufficient physical properties in the end product. Based on results from the SEM studies as well as water retention capacities and particle size distribution, hemp was expected to be a suitable structural component in plant based compost (Dresbøll and Magid, submitted; Dresbøll and Thorup-Kristensen, submitted B). Although a large part of the hemp stem is readily degraded, the remaining parts seem to be suitable as they consist of a large amount of helical secondary walls, which probably will enhance the capillarity of the compost by connecting larger particles. In addition, external bast fibres will also increase the continuity of the media. Wheat and Mischanthus straw are both degraded to much more rigid parts, containing a large amount of small particles and long almost intact parts of straw in contrast to the hemp particles having a more uneven surface and size of particles (Dresbøll and Thorup-Kristensen, submitted B). Thus, plant species having high fibre content might be good choices when producing compost as a growing medium. Clover-grass was chosen as nutrient rich source in the studies by Dresbøll and Thorup-Kristensen (submitted A) since it is an easily available nutrient supply, readily mixable and has really good nutritional properties. Despite the structural qualities of hemp there are certain obstacles towards using hemp. When the material is harvested fresh and cut immediately the nitrogen content can be high, which will increase decomposition and make the material unstable (Dresbøll and Thorup-Kristensen, submitted B). However, if hemp is not used until after the retting process the shredding of the material becomes complicated as the bast fibres are very strong and it takes special thresh mills to cut the fibres. After this processing the fibres have a 'wool-like' structure which makes it difficult to mix with other materials. This however, might be overcome by harvesting the hemp late in the season and shredding it without going through the primary retting process.

A less scientific approach to the choice of material for plant-based substrate is an economical approach. In order to make compost applicable in practice, it has to be able to compete with the prices on peat. Clover-grass is grown in large quantities in Denmark and could be used without large expenses. This is more uncertain when it comes to the structural elements. Wheat straw could be used at low expenses but results showed that wheat straw alone was not suitable as growing medium as stability and water retention capacities were not satisfactory (Dresbøll et al., in prep; Dresbøll and Thorup-Kristensen, submitted A). Growing hemp and Mischanthus straw would be somewhat more costly; however, hemp is becoming a more common crop for different purposes, the leaves can be used for tea, the seeds for oil production for cosmetics and fibres for robe, clothes and insulation. Both fibres and stem could though be produced without being much more expensive than peat. Mischanthus straw is grown as an energy crop and to some degree as material for thatched roofs, and it is the same part of the plant that should be used for composting. As Mischanthus in addition is very costly to establish, this might bring too high expenses on the growers. Thus, hemp-based compost, maybe combined with wheat straw to keep expenses down, is believed to be an economically and structural alternative to peat.

4. Conclusion and future directions

Composting is a complex process influenced by many different parameters. Much research has been conducted on various topics on composting. However, many of the studies are very specific, describing composting of special materials which makes generalisations complicated. The studies comprised in the present dissertation intended to investigate some basic processes during composting, both regarding nutritional quality and structural changes. From the nutritional studies it was concluded, that:

- Postponing the addition of some of the nutrient rich material affected the mineralisation pattern, resulting in more plant available N after 7½ weeks of composting.
- Total losses of mass, C or N were not affected significantly by the delayed addition of some of the clover-grass hay. However, temperature and pH differed due to the postponed addition.
- There seemed to be a critical N addition below which net mineralisation was not obtained during the composting period.
- After 24 weeks of incubation an effect of postponed addition was shown in the nutrient-poor compost with significant higher content of mineralised nitrogen when addition of part of the clover-grass hay was delayed 3 weeks during the previous composting period.
- The altered mineralisation pattern due to the postponed addition is believed to be a result of changes in the microbial communities.

The exact mechanisms behind the changes, due to the postponed addition, were not revealed but it is conceivable that implementation of molecular methods determining the microbial communities present, could give a more profound understanding of the microbial changes taking place.

Structural properties of the materials used for compost based growing media are of immense importance for horticultural purposes. The studies comprised in this dissertation revealed the discrepancies in the decomposition rate and extent between different straw materials. From the structural studies it was concluded, that:

- Compost based solely on wheat straw and clover-grass hay might not be sufficiently stable.
- Decomposition of straw material during composting is not solely dependent on the content of recalcitrant chemical compounds and nutrients but more importantly on the anatomical arrangement of the tissue, as penetration of microorganisms to cells within the straw material is impeded by the anatomy of the straw.
- Differences in anatomical arrangement of lignified tissues in monocots and dicots affect decomposition, and the structural quality of the residues left after decomposition.
- Structure of what is left after decomposition is important when choosing plant material for compost as a growing medium.
- Particle size was shown not to be a good indicator of the physical properties of composted plant residues.
- No differences in particle size distribution was found between composts based on three different straw materials; wheat, hemp and Mischanthus straw.
- Hemp based compost had a higher water retention than the other plant based composts and the geometry of the particles were more irregular.
- Compost based on hemp straw is expected to be a realistic alternative to the use of peat.

Basic knowledge of the actual decomposition of various cell types is a prerequisite for successful production of a growing medium, but also the physical arrangement within the final compost medium is important. Thus, future work should focus on how these particles of degraded material are distributed within the compost matrix and how this affects the physical properties of the medium. In addition, focus on how root growth is affected by the structural properties of the medium would be useful in the optimisation of growing media for organic greenhouse production.

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Appendix I

Delayed nutrient application affects mineralisation rate during composting of plant residues

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Submitted to Bioresource Technology

Delayed nutrient application affects mineralisation rate during composting of plant residues

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Abstract

We investigated the N turnover in compost based on wheat straw and clover-grass hay. Postponing the addition of some of the nutrient rich material was expected to influence the microbial succession and thereby the mineralisation pattern. After the initial bacterial degradation of soluble carbohydrates, more recalcitrant compounds are decomposed by less N demanding fungi. Thus, when the additional clover-grass hay was added less immobilisation was anticipated and more mineralised N would be available. After 7½ weeks of composting almost twice as much N was mineralised after the postponed addition. The delayed addition resulted in a second temperature peak and a decline in the pH. Despite the altered conditions no significant effect was observed on the weight loss or loss of C and N. In conclusion, compost processes can, in a simple way, be affected by delayed substrate application leading to a higher nutrient availability without altering other parameters significantly.

Keywords: Composting; Nitrogen mineralisation; Growing medium; Plant residues; Decomposition

1. Introduction

Composting experiments have been performed intensively during the last decades. Primarily the studies have focused on rural and urban wastes, often with the aim of reducing volume and avoiding nutrient losses (Witter and Lopez-Real, 1988; Martins and Dewes, 1992; Sánchez-Monedero et al., 2001). More recently focus has also been on composting of plant residues to produce growing media (Jensen et al., 2001; Prasad and Maher, 2001; Garcia-Gomez et al., 2002). Major topics in the composting research have been process control as well as characterisation of maturity or stability criteria. Control of a composting process and the properties of the end product can be achieved by at least two different strategies. One strategy is to adjust process parameters, such as moisture level, temperature or oxygen content (Beck-Friis et al., 2001; Smårs et al., 2002). Another is to alter the starting conditions by changing the composition or type of material used so that C/N ratio or fibre composition is changed (Eklind and Kirchmann, 2000a and 2000b; Eiland et al., 2001). A third strategy,

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which to our knowledge has not yet been subject to experiments, is to influence the composting process by altering the time of addition of parts of the material to be composted; normally all the material to be composted is included right from the start.

Nitrogen has often been recognised as a limiting factor for microbial growth and activity during the decomposition of plant residues (Recous et al., 1995), especially in materials with a high C/N ratio such as wheat straw. However, experiments on the effect of additional N supply to decomposing plant residues showed different results, ranging from positive to negative effects on the decomposition rate (Fog, 1988). Resource quality, microclimatic conditions and decomposer efficiency are major factors regulating composition and activity of decomposer communities and hence the process of decomposition and nutrient release (Neely et al., 1991; Ågren et al., 2001). Thus, the effect of added N on decomposition may depend on the plant material as degradation is influenced by nutrient content and anatomical structure of the material. Parameters such as N source and the time scale of the decomposition process are also influencing the effect of added N.

Recous et al. (1995) found that the ratio N immobilised-to-C mineralised decreased with time, and suggested that there is a high N demand during the first stages of decomposition when soluble and easily degradable C compounds were mineralised while the N demand was lower when the more recalcitrant C compounds were decomposed.

Since much C from plant residues such as straw materials is only slowly available to microorganisms leading to low growth efficiency, a limited amount of N may be required during decomposition, and recycling of N may then be adequate to meet the N requirements (Bremer et al., 1991). Microorganisms, especially fungi, have a considerable capacity to adapt to N deficient conditions. A large amount of N initially could consequently result in immobilisation. This greater N immobilisation may depend on 1) synthesis of microbial biomass with a lower C/N ratio; 2) higher N losses; or 3) reduced N mineralisation or re-mineralisation, which may have been related to reduced microbial activity (Bremer et al., 1991).

When composting material with the purpose of creating a growing medium it is important to understand the mineralisation and immobilisation processes as nutrient release is controlling plant growth. Availability of nutrients from organic composts is often limited despite a high initial nutrient input, and considerable nutrient losses frequently occur during the composting period, primarily due to gaseous emissions. As nutrients are a limited resource in organic production a more efficient nutrient use is desirable. Many horticultural plants are very nutrient demanding and compost used as fertilisers should provide a high nutrient level from the start. We hypothesised that such high efficient composts could be prepared by splitting the addition of the nutrient rich material during the composting process. The first addition at the start of the composting process should be sufficient to support the turnover of the readily available carbohydrates. The remaining nutrient rich material should be added later in the process when the turnover of the wheat straw would already be proceeding. Decomposition of the newly added material would then result in less N immobilisation compared to compost produced by a single addition at the beginning of the process.

The objective of this study was to test this hypothesis, by comparing turnover and N release in composts prepared in the above mentioned way with composts prepared with all the material initially.

2. Methods

2.1. Experimental design and materials

Compost was made of wheat straw as structural component and clover-grass hay as a nutrient rich component. The wheat straw was air dried after harvest and had a C/N ratio of approximately 100, whereas the clover-grass hay was oven dried after harvest and shredded into pieces of <20 mm. The C/N ratio of the clover-grass hay was 15.

Table 1. The setup for experiment I and II including amounts of material added to each compost box in the different treatments, and the initial C/N ratio and water content of the mixtures.

	Wheat straw	Clover-grass initial	Clover-grass 3 weeks	C/N	Water
	kg				(%)
Experiment I					
Treatment 1 (0): All material initially	32	48	0	25	78
Treatment 2 (2/3): 1/3 clover-grass initially, 2/3 after 3 weeks	32	16	32	38	79
Treatment 3 (5/6): 1/6 clover-grass initially, 5/6 after 3 weeks	32	8	40	50	77
Experiment II					
Treatment 1 (0): All material initially	35.5	21.5	0	35	54
Treatment 2 (3/4): ¼ clover-grass initially, ¾ after 3 weeks	35.5	5.5	16	60	52

Two experiments were set up as summarised in Table 1. Experiment I had three different treatments with three replicates in each. Treatment 1 was a mixture of clover-grass hay and wheat straw giving a C/N ratio of 25. Treatment 2 had only one third of the clover-grass hay from the beginning and thus an initial C/N ratio of 38. Treatment 3 had even less, only one sixth of the clover-grass hay initially, which resulted in a C/N ratio of 50. After three weeks of composting, when temperature had decreased to 20ºC indicating less microbial activity in treatment 2 and 3, supplemental clover-grass hay was added in these treatments. Hence, in the end all three treatments had received the same amount of clover-grass hay and wheat straw in total.

In experiment II only two different treatments with three replicates were compared as no significant differences was found between treatment 2 and 3 at the end of experiment I. The N content was reduced in this experiment as compost from experiment I initially were too nutrient rich as a growing medium. Treatment 1 was a mixture of wheat straw and clover-grass hay giving a C/N-ratio of 35. Treatment 2 contained only one forth of the clover-grass hay initially resulting in a C/N ratio of 60. Three weeks later the remaining clover-grass hay was added.

The wheat straw was cut in pieces of <10 cm and the substrates were mixed in a compost mixer and watered to a water content of approximately 70%. Leaching losses during mixing were avoided by placing some of the straw material beneath the mixer to absorb runoff. After mixing, the straw material was added to the compost boxes. Additional water was added whenever necessary during the composting time to keep the water contents above 50%. The composting experiments were performed in wooden boxes measuring 0.7 x 1.0 x 1.2m (height x width x length) and containing 800 L. Heat loss was minimised by insulation with glass-wool mats and the boxes were passively aerated by heat convection. Total composting time was 7½ weeks in experiment I, and 8 weeks in experiment II. After three weeks the compost of both experiments was turned and watered in the compost mixer and the supplemental clover-grass was added.

2.2. Sampling

Every 1½-week samples were taken for analysis of water content, mineralised N, total N and C content, and pH in experiment I. In experiment II sampling was conducted every week. Five samples of approximately 100g were collected from each box in experiment I, pooled and mixed. Subsamples were taken from the pooled sample for each of the analyses. In order to improve sampling techniques ten smaller samples of approximately 5g were taken from each compost box at a depth of 0.2-0.5 m and pooled for each analysis in experiment II. The entire sample was used for the analysis. Samples were collected randomly from the boxes except for the peripheral zone of 0.1 m in both experiments. At the beginning, after three weeks and at termination of the experiments all boxes were weighed to determine the total weight loss. Temperature was measured continuously in the centre of the composting boxes using standard acid proof stainless steel Pt-100 probes connected to a data logger (Datataker DT500).

2.3. Physical and chemical analysis

Water content was determined by weight loss of compost samples, which were oven dried at 80°C for 24 hours. Total N and C were measured by dry combustion of dried and finely ground samples with an automated N-C analyser interfaced with an isotope mass spectrometer (Carlo Erba, EA 1108). Samples were acidified by pouring 60 ml 0.1M HCl over the entire sample before drying to avoid NH_4^+ losses.

Compost samples (20g fresh weight) were analysed for NH_4^+ and NO_3^- content in a 2M KCl extract (compost: solution ratio 1:10) followed by shaking for 45 minutes and centrifugation. The supernatant was filtered through Advantec 6 (Frisenette Aps.) filters and stored frozen. As the extracts were dark coloured by organic acids, they were cleared by shaking the extract with active C for 15 minutes and filtered (0.45µm pore size) to avoid interference when measured spectrophotometrically. The analysis for NH_4^+ and NO_3^- content was conducted by standard colorimetric methods using flow-injection analysis (FIA) (Keeney and Nelson, 1982). Ammonium was measured by high pressure liquid chromatography (HPLC) in experiment I. pH was measured in a solution of compost (20g fresh weight) and water in a ratio of 1:5.

2.4. Statistics

The results were calculated as an average of three replicates of each treatment. Data were log transformed to obtain homogeneity of variance and analysed with the GLM procedure of the SAS statistical package (SAS Institute Inc., Cary, NC, USA)

3. Results

3.1 Process parameters

3.1.1. Temperature

In both experiments the compost in treatment 1 showed a similar temperature development pattern (Fig. 1a and b). Initially a short mesophile phase lasting one or two days with temperatures below 40°C was observed followed by a thermophile phase with temperatures above 70°C lasting 2-3 days. Hereafter the temperature declined to mesophile temperatures again, that is less than 40°C. After addition of the supplementary clover-grass hay in treatment 2 and 3 the temperature increased again to 70°C followed yet again by a fast decline. Treatment 1 in both experiments did not show this second temperature rise (Fig. 1a and b).

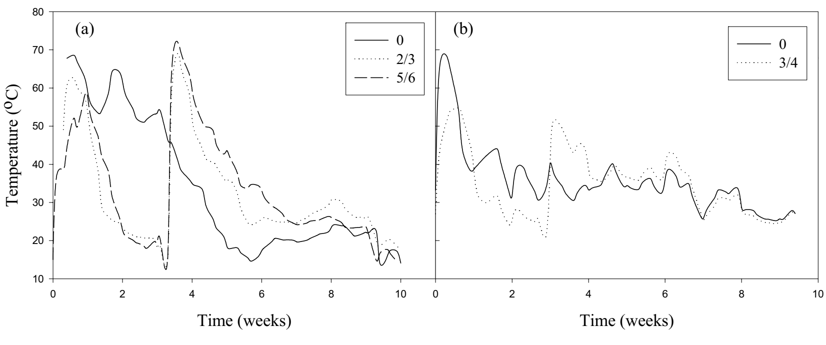

Fig. 1. Temperature development during composting in (a) experiment I: treatment 1: all material initially (solid line), treatment 2: 1/3 clover-grass hay initially, 2/3 after 3 weeks (dotted line), and treatment 3: 1/6 clover-grass hay initially, 5/6 after 3 weeks (broken line). (b) shows temperature development in experiment II: treatment 1: all material initially (solid line), and treatment 2: ¼ clover-grass hay initially, ¾ after 3 weeks (dotted line).

3.1.2. Compost pH

pH varied between 7.6 and 8.9 in treatment 1 of experiment I and the highest values were found after 3-4 weeks (Fig. 2a), where also the NH_4^+ concentration was high. Towards the end of the experiment the pH declined to 8.4. Treatment 2 and 3 followed the same pattern with the exception that after 3 weeks when the additional clover-grass hay was added the pH declined to 6.6-6.7. In treatment 1 of experiment II pH varied between 7.3 and 8.9 whereas pH in treatment 2 varied between 7.5 and 8.5. In both treatments an increase in pH was observed during the first half of the composting period. Towards the end of the composting period a small decrease was observed in treatment 1 (Fig. 2b).

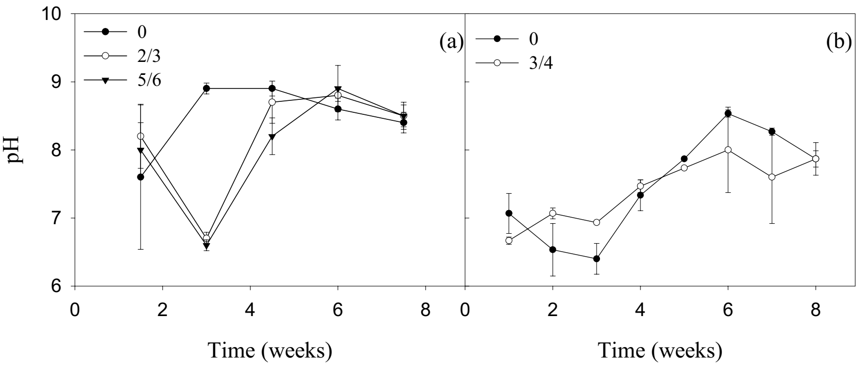

Fig. 2. Changes in pH during composting of wheat straw and clover-grass hay in (a) experiment I: treatment 1: all material initially (●), treatment 2: 1/3 clover-grass hay initially, 2/3 after 3 weeks (○), and treatment 3: 1/6 clover-grass hay initially, 5/6 after 3 weeks (▼) and (b) experiment II: treatment 1: all material initially (●), and treatment 2: ¼ clover-grass hay initially, ¾ after 3 weeks (○). Results are the mean of three replicates and bars show the standard deviation.

3.1.3 Weight loss

After three weeks of composting, before additional clover-grass was added, no significant differences were found in weight losses between the treatments in experiment I (44-45% of initial weight). In experiment II weight losses after three weeks were only half the losses in experiment I and no significant difference between the treatments was neither found here (Table 2). After 7½ weeks weight losses in all treatments were about 61-63% of initial weight in experiment I, whereas the weight losses varied between the two treatments in experiment II after 8 weeks with a loss of 48% in treatment 1 compared to a loss of 54% in treatment 2 (Table 2).

3.2. Carbon and nitrogen loss

Total loss of C was determined from the changes in compost total C content over the experimental periods. After three weeks of composting no significant differences were observed between the treatments in neither of the experiments. After 7½ weeks the C losses did not vary significantly between the three treatments in experiment I whereas the difference

Table 2. Weight and carbon losses after three weeks of composting and at the end of the composting experiments, and N losses at the end of the experiments. Results are means of three replicates. Standard deviations are shown in parentheses.

	3 weeks		Final		
	Weight loss (%)	C loss (%)	Weight loss (%)	C loss (%)	N loss (%)
Experiment I					
Treatment 1: All material present initially.	46 (6)	50 (7)	61 (6)	60 (6)	4 (16)
Treatment 2: 1/3 clover-grass initially, 2/3 after 3 weeks.	45 (5)	52 (6)	63 (5)	63 (6)	12 (10)
Treatment 3: 1/6 clover-grass initially, 5/6 after 3 weeks.	44 (7)	52 (8)	61 (1)	62 (2)	9 (3)
Experiment II					
Treatment 1: All material present initially.	16 (5)	24 (11)	48 (9)	54 (3)	30 (24)
Treatment 2: ¼ clover-grass initially, ¾ after 3 weeks.	26 (8)	32 (3)	54 (5)	58 (5)	22 (11)

between the two treatments in experiment II after 8 weeks was more pronounced (Table 2). As observed in the weight losses, the C losses were significantly higher in experiment I compared to experiment II.

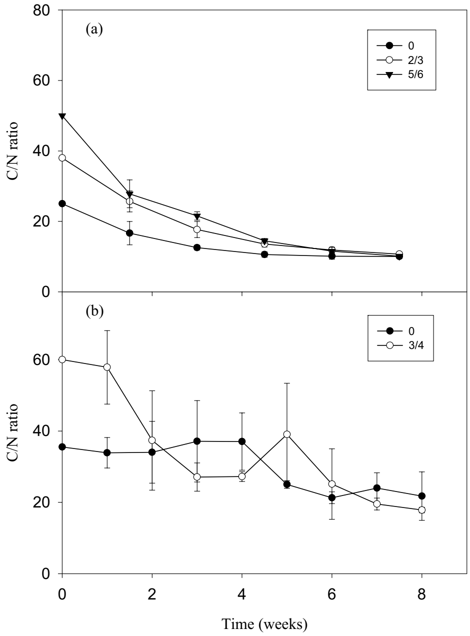

Fig. 3. Changes in C/N ratio during composting of wheat straw and clover-grass hay in (a) experiment I: treatment 1: all material initially (●), treatment 2: 1/3 clover-grass hay initially, 2/3 after 3 weeks (○), and treatment 3: 1/6 clover-grass hay initially, 5/6 after 3 weeks (▼) and (b) experiment II: treatment 1: all material initially (●), and treatment 2: ¼ clover-grass hay initially, ¾ after 3 weeks (○). Results are the mean of three replicates and bars show the standard deviation.

Nitrogen losses were determined as the mass balance of N and reflected both N gaseous emissions and N leaching. As the water content was regulated carefully throughout the composting period the losses due to leaching were minimal. Nitrogen losses in experiment I was low with less than 13% of total N lost during the composting period. In experiment II higher N losses were observed in both treatments with up to 30% losses (Table 2). The percentage of C in the compost of both experiments was quite constant around 46% during the composting period although there was a slight tendency to a reduction in the C content (results not shown). The percentage of N increased from 2.8 to 4.6% in treatment 1 of experiment I whereas it increased from 1.7-1.8 % to 4.6% in the other treatments. In experiment II the increase was less steep from 1.3 to 2.2% in treatment 1 and from 0.8 to 2.5% in treatment 2. These results are reflected in the C/N ratio (Fig. 3).

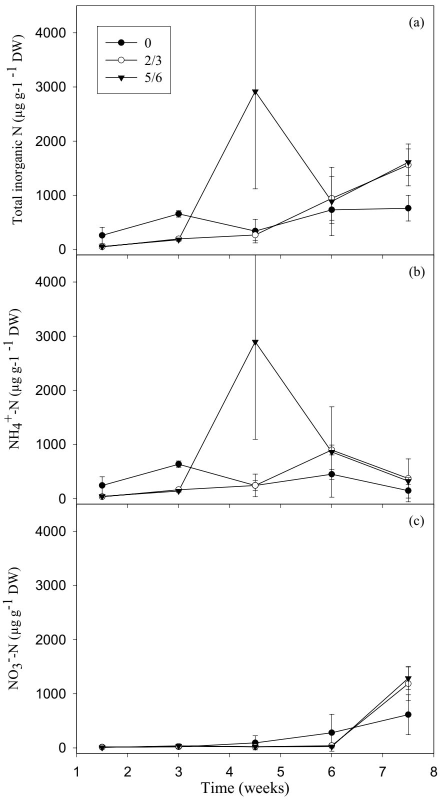

Fig. 4. Mineralisation pattern during composting of wheat straw and clover-grass hay in experiment I: (a) total mineralised nitrogen, (b) ammonium content, and (c) nitrate content. Treatment 1: all material initially (●), treatment 2: 1/3 clover-grass hay initially, 2/3 after 3 weeks (○), and treatment 3: 1/6 clover-grass hay initially, 5/6 after 3 weeks (▼). Results are means of three replicates and bars show the standard deviation.

3.3. Nitrogen mineralisation

Postponing the addition of some of the nutrient rich material had a significant effect on the timing and extent of the mineralisation processes in experiment I. When all the clover-grass hay was added initially the NH_4^+ content increased during the first weeks, and declined afterwards (Fig. 4b). The NO_3^- content remained low for the first three weeks, followed by a steady increase (Fig. 4c). Postponing the addition of some of the clover-grass hay resulted in an altered mineralisation pattern. The NH_4^+ content increased slightly during the first three weeks followed by an increase when the rest of the clover-grass hay was added (Fig. 4b). After five to six weeks a decline in NH_4^+ was observed. At the same time a steep increase in NO_3^- content was seen after six weeks of no NO_3^- production (Fig. 4c). At the end of the experiment the total mineralised N content was significantly higher ($p<0.05$) after the postponed addition (Fig. 4a). About 3.5% of the initial total N was mineralised after 7½ weeks in treatment 2 and 3 whereas only about 1.6% of initial total N was mineralised in treatment 1.

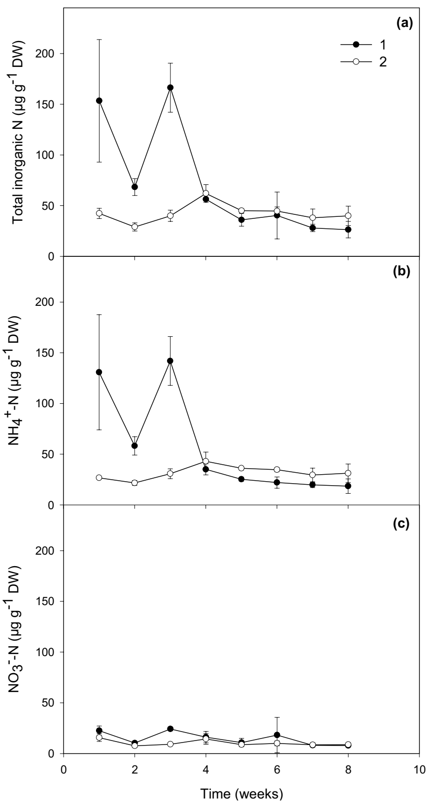

Fig. 5. Mineralisation pattern during composting of wheat straw and clover-grass hay in experiment II: (a) total mineralised nitrogen, (b) ammonium content, and (c) nitrate content. Treatment 1: all material initially (●), and treatment 2: ¼ clover-grass hay initially, ¾ after 3 weeks (○). Results are means of three replicates and bars show the standard deviation.

In experiment II only a small amount of NH_4^+ was detected during the composting period, whereas practically no NO_3^- was found (Fig. 5b and c), hence after 8 weeks only 0.15% of the initial amount of total N was mineralised.

4. Discussion

4.1. Process parameters

In general, maximal mass reduction is desirable during composting of waste products. However, the objective of these experiments was to produce a growing medium while mass reduction was not a goal. Mass losses during composting of different materials such as deep litter or household waste are found in a wide range from about 40 to 80% (Sommer and Dahl, 1999; Eklind and Kirchmann, 2000b). The mass losses in these experiments were found within the lower end of this range. Mass losses are most importantly dependent on composting time and type of material (Eklind and Kirchmann, 2000b). To obtain even lower mass losses more stable structural materials could be considered.

Total C losses resembled losses normally seen during decomposition of plant material (Bremer et al., 1991). It could have been expected that the mass and C losses would be lower in treatment 2 and 3 in experiment I with the delayed addition of nutrient rich material, since the decomposition was expected to proceed more slowly but steadily after the initial decomposition of the readily available carbohydrates. However, no decrease or even an increase of the mass and C loss was seen in treatment 2 and 3 compared to treatment 1. This could be explained by the higher microbial activity in treatment 2 and 3 when the supplementary clover-grass hay was added. The second peak of microbial activity in treatment 2 and 3 was detectable on the temperature curves, as temperature exceeded 70°C again when the extra clover-grass was added. Normally a small increase in temperature occurs when the compost is turned due to better aeration and humidity conditions. This was not observed in these experiments probably due to sufficient aeration in the compost as a result of the experimental set-up and the relatively small size of the composting boxes.

pH of both experiments tentatively followed the same pattern. During the first 3-4 weeks pH increased. This is normally observed during the initial part of the composting process when organic N is mineralised, as this is a proton assimilating process (Beck-Friis et al., 2003). The following decrease in pH coincided with NO_3^- production as the nitrification process releases protons.

4.2. Maturity parameters

During composting the C/N ratio decreased to around 10 in all three treatments of experiment I, which indicated the biological stability of the composts (Bernal et al., 1996). The C/N ratio can be used as a compost maturity parameter implying a stable organic matter content and absence of phytotoxic compounds (Bernal et al., 1998). Another maturity parameter is the ratio between NH_4^+-N and NO_3^- -N as decreasing amounts of NH_4^+-N combined with increases in NO_3^--N concentrations towards the end of composting suggests that intensive biological decomposition has been completed (Paré et al., 1998). This shift in inorganic N was seen in experiment I. In experiment II on the other hand the C/N ratio never declined to less than 20 and no NO_3^- was produced confirming that decomposition became N limited.

4.3. Nitrogen mineralisation

The mineralisation in treatment 1 in experiment I followed a pattern often observed during decomposition in compost (Eklind and Kirchmann, 2000b). Initially the NH_4^+ content increased due to the microbial decomposition of readily available N rich compounds. After the first three weeks the NH_4^+ content declined, most likely due to oxidation of NH_4^+ to NO_3^-. During the microbially very active initial phase NH_4^+ can be accumulated, which can result in an elevation of the pH. This combination of high pH, high NH_4^+ concentrations and high temperatures promote NH_3 volatilisation and the highest ammonia losses occur during this phase (Witter and Lopez-Real, 1987; Martins and Dewes, 1992, Beck-Friis et al., 2003). Despite the presence of conditions permitting ammonia losses the total N losses were low, 4-29% of initial N content with no significant difference between the two experiments. Variation between replicates was high, which probably was due to uncertain determination of the dry matter content of the total amount of compost in the boxes. Compared to what is normally seen during composting these losses were relatively small. During composting of animal manure, household waste, and other waste products losses of at least 50% may be observed (Witter and Lopez-Real, 1988, Martins and Dewes, 1992; Eklind and Kirchmann, 2000b). The low N losses in these experiments could partly be explained by the experimental set-up as the compost was not rotated and aerated as much as compost in reactors (Eklind and Kirchmann, 2000a; Beck-Friis et al., 2003) or in heaps (Martins and Dewes, 1992; Sommer, 2001). Additionally, composting time was short compared to other experiments, although this might have a minor effect on the N losses, as the main part is lost during the first weeks of composting (Witter and Lopez-Real, 1987; Sommer, 2001).

Nitrate contents were not measurable until after the initial three weeks of composting. The nitrifying bacteria oxidizing NH_4^+ to NO_3^- show an optimal temperature of about 40°C (Prescott et al., 1990), thus the high temperatures during the initial phase probably inhibited nitrification as these bacteria were killed. Nitrifying bacteria probably survived in the peripheral zone of the composting boxes where the temperature was not as high as the centre of the compost pile. Nitrate content in the compost increased after the temperature decreased and the compost was turned, suggesting that nitrifying bacteria were mixed into the compost. In treatment 1 of experiment I the NO_3^- content continued to increase steadily throughout the remaining composting time.

Postponing the addition of some of the nutrient rich material altered the mineralisation patterns significantly in experiment I. During the first three weeks the NH_4^+ content was low as no net mineralisation occurred, probably because inorganic N was immobilised during the degradation of soluble and easily degradable carbohydrates. Recous et al. (1995) observed a decrease in the ratio of N immobilised to C mineralised with time confirming the initial high

N demand. When the supplemental clover-grass hay was added after three weeks an increase in NH_4^+ content was observed, indicating that the organic N from the clover-grass hay amendment was mineralised and N immobilisation was lower than in treatment 1. These results support the hypothesis that a limited amount of N is needed initially in the decomposition of the readily available carbohydrates of the straw material (Bremer et al., 1991). Usually bacteria degrade the soluble compounds during the initial phases of decomposition whereas fungi with a higher C/N ratio decompose more recalcitrant compounds (Recous et al., 1995; Klamer and Bååth, 1998). The fungal/bacteria index increases during the decomposition of material with a high initial C/N ratio. The same phenomenon occurs during the initial phases of composting (Eiland et al., 2001) confirming the fungal dominance in degrading recalcitrant compounds. When the additional N was added the readily available carbohydrates were presumably already degraded and less N demanding fungi dominated the decomposition. Thus, when the N was mineralised from the supplemental clover-grass hay it was not re-immobilised by the microbial population to the same degree as when all clover-grass hay was added initially. Therefore the delayed addition of clover-grass hay resulted in a higher total release of inorganic N during the experimental period. The NO_3^- content remained, however, low for three weeks more than in treatment 1. This can be explained by the lower NH_4^+ production during the initial three weeks compared to treatment 1 but also by the fact that the extra addition increased temperatures to above 70°C again and thereby postponed the growth of nitrifying bacteria. The steep increase in NO_3^- after six weeks resulted in the NO_3^- content being twice as high as in treatment 1 at the end of the composting process.

When used as a growing media for lettuce (*Lactuca sativa* L.) root growth was inhibited during the first few weeks in the treatments with postponed nutrient application in experiment I probably due to the high NH_4^+ content (unpublished results). Due to these results the initial N level was reduced in experiment II resulting in a N level, which probably was so low that almost no net mineralisation occurred. Only a small net production of NH_4^+ occurred during the first three weeks in treatment 1 where after immobilisation was detected (Fig. 5a and b). During the first three weeks treatment 1 of experiment II was comparable to treatment 2 of experiment I, having similar initial C/N ratios. Hence, presumably sufficient N was available for the initial bacterial decomposition of soluble compounds in treatment 1 of experiment II. The increase in temperature as well as the C and weight losses indicated considerable microbial activity. During decomposition of plant material in soil Recous et al. (1995) observed that if mineral N was not available the C decomposition decreased but it did not stop. Despite the low N content in treatment 1 of experiment II the C mineralisation proceeded throughout the experimental period, suggesting that the initial low N content could have altered the microbial succession. Decomposition might have been dominated by fungi which may have the ability of effective remobilisation and transfer of N to actively growing parts (Cowling and Merrill, 1966). As no supplemental clover-grass hay was added in treatment 1 of experiment II no considerable net N mineralisation was observed after the initial three weeks.

Conclusions

In conclusion, postponing the addition of nutrient rich material affected the mineralisation pattern resulting in more plant available N after 7½ weeks of composting. Total losses of mass, C or N were not affected significantly by the delayed addition. This suggests that without altering amount or type of material to be composted, mineralisation can be managed by simple methods, which can be of great importance when growing plants with a high initial N demand. There seemed, however, to be a critical N addition below which net mineralisation was not obtained. When using compost exclusively as a growing medium a high nutrient availability is not sufficient, as stability and structure, as well as the absence of inhibitory compounds also are important. These composts based solely on wheat straw and clover-grass hay might not be sufficiently stable and could experience structural difficulties such as low water retention capacity. It might be difficult to produce compost both with high nutrient release and stability. Combining composts of different properties might resolve these problems and could be a subject for future research on organic growing media.

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Appendix II

Structural changes of plant residues during decomposition in a compost environment

Dorte Bodin Dresbøll, Jakob Magid

Submitted to Bioresource Technology

Structural changes of plant residues during decomposition in a compost environment

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Abstract

We investigated the actual degradation of plant material during composting by qualitative methods using Scanning Electron Microscopy (SEM) combined with quantitative chemical methods. Decomposition of Mischanthus (*Mischanthus oogiformis* L.), hemp (*Cannabis sativa* L.) and wheat (*Triticum aestivum* L.) straw was observed by placing litterbags containing these materials in compost piles. Hemp and Mischanthus straw were more stable than the wheat straw, but the two materials differed in the way they were degraded despite similar chemical composition. Hemp straw was broken down in more flexible structures compared to the rigid breakdown of Mischanthus straw. We concluded that the anatomical arrangement of the tissue is just as an important part of the decomposition rate as the content of recalcitrant compounds. Thus, when using composted plant materials as growing medium the choice of material must depend not only on nutritional quality but also on structural quality. This study indicates that hemp material might be a good structural component in a compost to be used as a growing medium.

Keywords: plant residues; decomposition; compost; growing medium; Scanning Electron Microscopy; anatomical arrangement

1. Introduction

Peat bogs in Europe have been subject to intensive exploitation for horticultural use during decades. Recently, focus on the environmental aspects of depleting these slowly renewable natural resources has increased. Thus, in both conventional and organic greenhouse production peat substitutes are desirable and composted plant residues could be an alternative. Using compost as a growing medium in greenhouse production poses certain requirements to the nutritional quality, the structure and the stability of the compost. Plant-based compost is

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often composed of two different types of plant material, a nutrient rich component and a structural component. In contrast to most composting processes where the focus is on waste management and mass reduction is attempted (García-Gómez et al., 2003; Ryckeboer et al., 2003), mass retention is desirable when composting plant residues for use as a growing medium. To find suitable structural materials we investigated how different plant materials are being decomposed, and how the various tissues are being degraded in time.

Decomposition of plant residues in soil or compost environments has been widely investigated. The extent of degradation can be determined by various methods. Mass loss, CO_2 emission, changes in C/N ratio and changes in hemicelluloses, cellulose and lignin content are some of the parameters often used to describe decomposition (Bremer et al., 1991; Recous et al., 1995; Eklind and Kirchmann, 2000; Sakala et al., 2000). Additionally, microbial diversity and activity are indicators of decomposition (Conti et al., 1997; Horiuchi et al., 2003). In general, these different methods focus mostly on the losses during decomposition and less on the plant parts remaining.

Decomposition rate is dependent on the chemical composition of the plant material and on the growth conditions for the decomposers (Fog, 1988). The chemical composition such as the nutrient availability and amount of recalcitrant compounds such as lignin has been examined extensively in relation to the decomposition rate. Another important parameter influencing the decomposition rate is the anatomical arrangement of the plant tissue as some cell types function as barriers towards microbial degradation (Wilson, 1990; Wilson and Mertens, 1995). In particular, lignified tissues function as a barrier to degradation. However, even lignified cells are degraded when plant material is ground or sliced finely thereby increasing the points of accessibility to the microorganisms (Wilson and Mertens, 1995). Thus, the spatial arrangement of lignified tissues is important when considering structural materials for growing media and is found to vary between plant types and species.

To ensure stability in compost, knowledge of lignin content and arrangement of lignified tissues are important but these factors do not necessarily determine the overall structure of compost based on plant residues. Besides being stable, another essential factor is water retention capacity, when compost is used as an alternative to peat. An important parameter influencing this could be the extent of decomposition and the size and appearance of the particles remaining after degradation. We expect that plants with flexible structures such as helical secondary walls and a high content of fibre cells could enhance water retention due to improved contact between the larger particles of the compost and thus enhance the capillarity. Plants having high fibre cell content might then be more suitable as a structural component. Many dicotyledonous species contain more fibre cells than monocotyledonous species as the xylary fibres make up a large part of the stem tissue (Mauseth, 1988). Additionally, presence of external bast fibres is more common in dicotyledonous species. When cut and decomposed these fibres may have a flexible structure improving the compost structure and thereby water retention capacity.

The objective of this study was to achieve a better understanding of structural quality and microbial decomposition during composting by examining extent and order of decomposition of plant tissues in three different structural materials. This was done by combining visual qualitative techniques such as scanning electron microscopy (SEM) with physicochemical measurements. The actual structural degradation of plant tissue during composting is not well known and information on rate and extent of decomposition of different plant materials and the physical quality of the material remaining after decomposition will enable optimisation of choice of structural material as part of a growing medium.

2. Methods

2.1. Experimental design and materials

Decomposition studies were conducted on air-dried straw of Mischanthus (*Mischanthus oogiformis* L.), hemp (*Cannabis sativa* L.) and wheat (*Triticum aestivum* L.). After harvest the hemp straw was left in the field and an initial decomposition process occurred. During this degradation the external bast fibres are released from the stem, this process is called retting. Materials were selected in order to produce compost of easily available plant residues; wheat straw was selected as it is cheap and especially easily available, Mischanthus straw as it previously has been used as structural element in compost, and hemp straw was selected due to the high fibre content. The materials were cut into pieces of 25 mm length. Further the materials were split vertically, the hemp and mischanthus straw in three partitions and the wheat straw in two partitions. Degradation of the wheat straw was examined at the basal and apical part of the stem, whereas the Mischanthus and hemp straw only was examined at the upper part of the stem. A minimum of 2 g of straw material was deposited in nylon mesh bags (mesh size 1.4 x 1.2 mm). The material was moistened and the litterbags were placed at 0.4m depths in 800 L wooden boxes measuring 0.7 x 1.0 x 1.2m (height x width x length) at the initiation of composting of a mixture of wheat straw and clover-grass hay.

The compost was made of wheat straw as structural component and clover-grass hay as a nutrient rich component. After harvest the wheat straw was air-dried and had a C/N ratio of approximately 100, whereas the clover-grass hay was oven dried after harvest and shredded into pieces of <20 mm. The C/N ratio of the clover-grass hay was 15. The wheat straw was roughly shredded; containing parts of up to 0.1 m in length and was mixed with clover-grass hay giving a C/N-ratio of 35.

The compost was mixed and watered to a water content of approximately 60%. Leaching losses during mixing were avoided by placing some of the straw material beneath the mixer to absorb runoff. After mixing this straw material was added to the compost in the box. Supplementary water was added whenever necessary during the composting time to keep the water contents above 50%. Heat loss was minimised by insulation with glass-wool mats and the boxes were passively aerated by heat convection. Total composting time was 8 weeks. After three weeks litterbags were carefully removed and the compost was turned and watered in a mixer. After the turning of the compost the litterbags were placed in the compost again.

2.2. Sampling

Litterbags (four replicates) were sampled after one, three and eight weeks of composting. The material in the litterbags was divided, a part of it was used for histological analyses and a part was oven dried at 70ºC for 24 hours and used for chemical analyses. As a reference fresh green material and un-decomposed air-dried material of the three straw types were collected, fixed and analysed histological.

2.3. Physical and chemical analysis

Material for histological analysis was fixed in FAA (formaldehyde, acetic acid, alcohol, 1:1:18). For scanning electron microscopy studies the material was dehydrated in a series of ethanol and acetone, critical point dried (EMS critical point drier), mounted on stubs and coated with gold in a sputter coater (Polaron SC7640). Specimens were examined in a scanning electron microscope (CamScan MaXim 2040S).

Remaining litterbag content was ground after drying and hot water-solubles, ADF, cellulose and lignin content was measured by C fractionation using a modified Van Soest method in a fibre analyser ($ANKOM^{220}$ fiber analyzer). Total N and C were measured by dry combustion of dried and finely ground samples with an automated N-C analyser interfaced with an isotope mass spectrometer (Carlo Erba, EA 1108).

2.4. Statistics

The results were calculated as an average of four replicates of each treatment; however the fibre content was calculated as the average of duplicates. The measurements from litterbags were truly independent over time and data were analysed using the GLM procedure of the SAS statistical package (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Weight loss

There were no significant differences in decomposition of the four different materials expressed as weight loss after one week. Initial losses were about 5 % of total dry weight (Fig. 1a). After three weeks the weight losses of 14 % at the apical part of the wheat straw differed significantly ($p<0.05$) from the basal part of the wheat straw, the hemp and Mischanthus straw, whereas no significant difference was observed between the basal part of the wheat straw and the hemp straw. At the end of the composting period, after 8 weeks, significant differences were observed between the apical and basal part of the wheat straw with weight losses of 34 and 25 % of initial weight and between the wheat straws and the two other materials ($p<0.01$). No significant difference was observed between the hemp and mischanthus straw, both having weight losses of 17%.

3.2. Nitrogen content and C-to-N ratios

During the composting period the C/N ratio for all four materials decreased (fig. 1b). The percentage of carbon in the material remained stable whereas the nitrogen percentage increased in all materials. The steepest increase in nitrogen percentage was seen in the apical part of the wheat straw as reflected by the steepest decrease in C/N ratio (Fig. 1b). Nitrogen content increased in all materials due to uptake from the surrounding clover-grass/wheat straw compost (Fig. 1c).

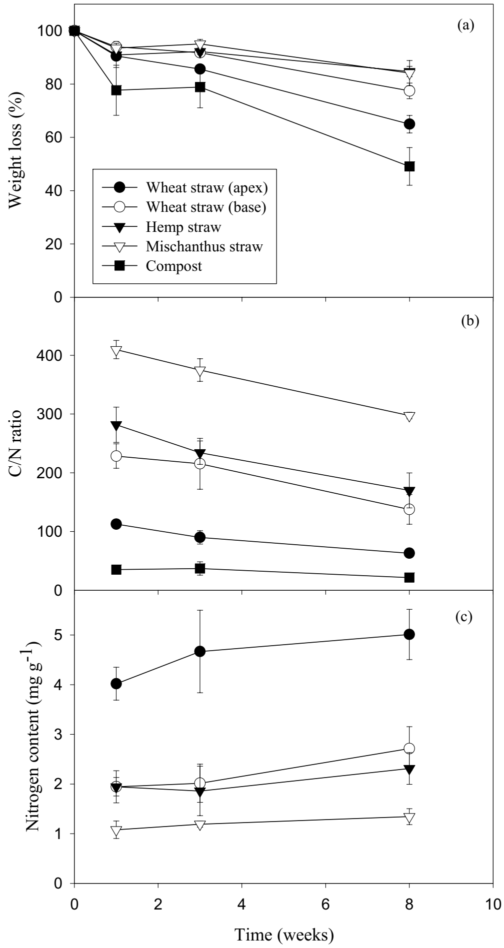

Fig. 1. a) The weight losses of straw of the apical and basal part of wheat, Mischanthus and hemp as well as losses in the surrounding compost. Results are means of four replicates and bars show the standard deviation. b) C/N ratio of the four different straw materials and the surrounding compost during the composting period. Results are means of four replicates and bars show the standard deviation. c) Nitrogen content during composting. Results are means of three replicates and bars show the standard deviation.

3.3. Temperature

These composting experiments showed a temperature development pattern similar to what is normally seen during composting (data not shown). Initially a short mesophilic phase was observed, lasting one or two days with temperatures below 40°C, followed by a thermophilic phase with temperatures above 70°C lasting 2-3 days. Hereafter the temperature declined to mesophilic temperatures again, that is less than 40°C.

3.4. Fibre content

When we started to use the ANKOM^{220} fiber analyzer, we realized that the results produced were unreliable and spurious. When finally the problems had been narrowed down we were left with little of our sample, only enough for duplicate measurements and that lends our results great uncertainty.

Therefore we will not give much credit to these here. We will only say that as seen in previous shorter-term decomposition experiments (Luxhøi et al., 2002) we did not observe a decrease in lignin in wheat and mischanthus straw relative to outset values. However results indicated a non significant decrease in lignin for hemp. Regrettably we cannot make claims to give a full account of the mass loss observed, by accounting for losses in fibre fractions – due to unreliable measurements

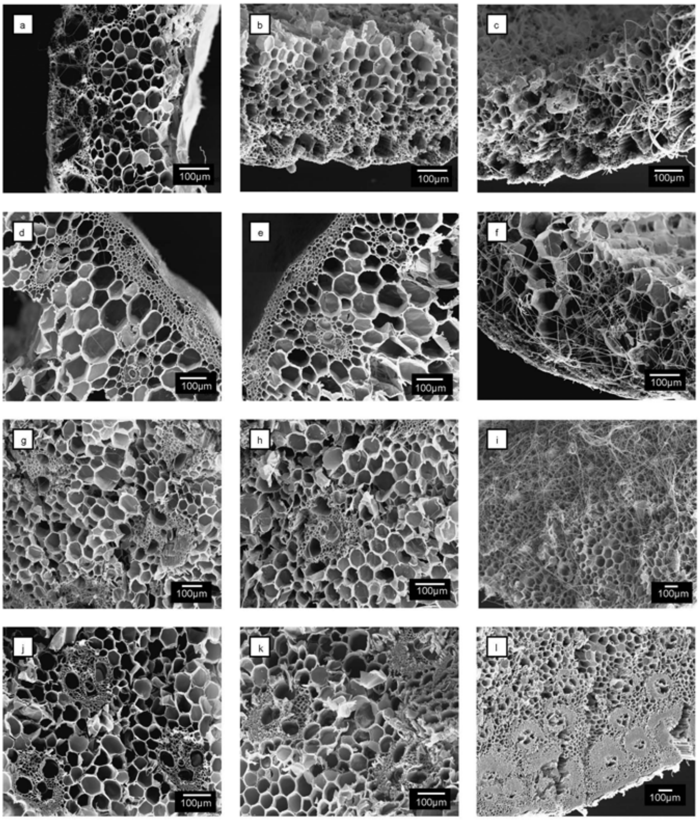

Fig. 2. a-c: Apical part of wheat straw after 1, 3 and 8 weeks of composting. Phloem and photosynthetic tissues has been degraded during the initial week of composting. After 8 weeks of composting the exposed surface is covered by fungal hyphae. d-f: Basal part of wheat straw after 1,3 and 8 weeks of composting. Phloem and photosynthetic tissues has been degraded during the initial week of composting. g-i: The exposed (exp.) surface of Mischanthus straw after 1, 3 and 8 weeks of composting. No clear degradation of the phloem was observed after 1 week of composting. After 3 weeks beginning degradation was observed and after 8 weeks the surface was covered by fungal hyphae. j-l: A freshly cut surface (not exp.) in the middle of the Mischanthus straw after 1, 3 and 8 weeks of composting. No degradation of the phloem was observed until after 8 weeks of composting.

3.5. Microscopic evidence

Visual differences in decomposition were apparent after one week. In the wheat straw decomposition was clearly observable as the phloem and photosynthetic tissue of the straw was degraded (Fig. 2a and 2d). After 3 and 8 weeks visible signs of degradation of the inner cortex was also seen in the wheat straw (Fig. 2b, c, f). Most of the material after 8 weeks of composting was covered by fungal hyphae (Fig. 2c, f, i). In contrast, the tissue in mischanthus straw did not reveal any degradation after one week (Fig. 2g).

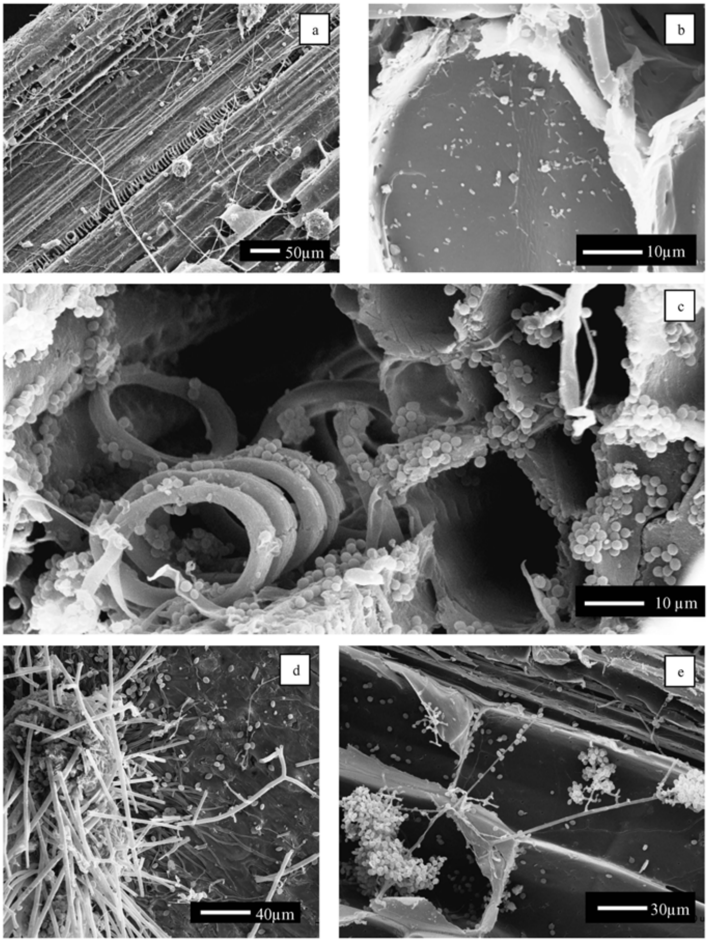

Fig. 3. a) The basal part of wheat straw covered by microorganisms after eight weeks of composting, b) Cell of mischanthus straw covered by different bacteria and fungal spores, c) Xylem vessel of the apical part of wheat straw after eight weeks of composting, the tissue is covered by bacterial colonies, d) A sporangium with spores and hyphae on the surface of the apical part of wheat straw after three weeks of composting, e) Fungal hyphae and spores in the internal structures of the basal part of wheat straw after one week of composting.

After three weeks of composting visual evidence of degradation was observed at the exposed surfaces of the mischanthus straw showing degradation of the phloem tissue (Fig. 2h). However, when divided at the middle of the piece of straw, no visible decay was observable at the cut surface (Fig. 2k). After 8 weeks, the phloem and photosynthetic tissue were extensively degraded in the mischanthus straw equally at the exposed surface and at the freshly cut surface (Fig. 2i and 2l). No difference in degree of degradation between the exposed surface and a freshly cut surface in the middle of the piece was observed at any time in the wheat straw. During the retting process and in the handling of dried hemp material the outermost layers of the stem were torn off, making it impossible to observe the phloem and photosynthetic tissue in this material.

3.5. Microorganisms

Microorganisms were observed on all materials after 1, 3 and 8 weeks. Except for hemp which had been retted no visible signs of microbial activity were observed on the fresh and air-dried material confirming that no decomposition had taken place before initiation of the composting process. After one week of composting, fungal spores and hyphae were seen on the wheat straw (Fig. 2a and 3e). After one week microorganisms were less numerous on the hemp and mischanthus straw, especially in the non-exposed tissues (Fig. 2j), reflecting their lower rate of decomposition. For the remaining composting period miscellaneous microorganisms were observed on the wheat straw. On the surface of the apical part of the wheat straw sporangiae surrounded by hyphae and spores were detected (Fig.3d) and the internal structures of the xylem element were presumably covered by bacterial colonies (Fig.3c). In hemp and mischanthus straw, only very little evidence of microorganisms was seen in the internal tissues whereas the exposed surfaces also revealed a broad variety of bacteria and fungi after 3 and 8 weeks of composting (Fig.4c, 3a and 3b).

3.6. Structure

The microscopic studies revealed the anatomical differences between monocotyledonous and dicotyledonous plants that resulted in differences during decomposition.

On a longitudinal section of the hemp it was possible to observe how the lignified reinforcing secondary walls of the tracheary elements of the xylem remained after degradation of primary walls and other cell components (Fig. 4b). In the wheat and mischanthus straw this reinforcement also remained but in these species it consisted of singular rings in contrast to the helical structures in hemp (Fig. 4a and 4d) and were broken down in more defined pieces than the hemp straw (Fig. 4e). The tissues of the monocotyledonous plants in these studies had a more rigid anatomical structure compared to the more flexible structure of hemp.

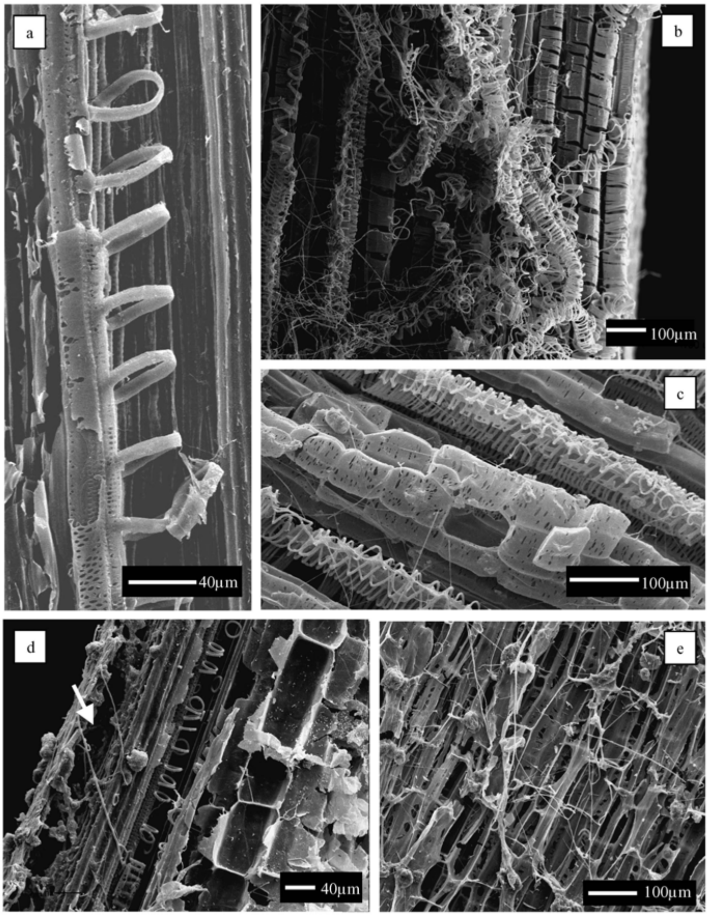

Fig. 4. a) Annular secondary wall reinforcements of Mischanthus straw after one week of composting, b) Helical secondary wall reinforcements of hemp straw after eight weeks of composting, c) Longitudinal section of hemp straw, exposed surface, after one week of composting, d) Longitudinal section of the apical part of wheat straw after eight weeks of composting, the cavity where the photosynthetic tissue has been degraded can be seen, e) The basal part of wheat straw after eight weeks of composting.

4. Discussion

4.1. Weight loss

The weight losses of the different straw materials were in the range normally seen. The wheat straws having the highest content of nitrogen and the lowest content of lignin also had the highest losses of mass. Especially the wheat straw apex was more readily degraded than the basal part. The fibre content was roughly the same in the basal and apical part of the wheat straw. However, as the photosynthetic tissue and vascular bundles compose a relatively larger part of the apical tissue and since these are the most nutrient rich tissues this would explain the higher degree of degradation in the apical part.

4.2. Lignin degradation

During the short composting time of only eight weeks no lignin degradation was expected. This was consistent with our observations in the wheat and Mischanthus straw but in the hemp straw a small but not significant decline in lignin content was seen.

Due to its recalcitrant nature, decomposition of lignin is a slow process performed only by few species, mainly white-rot fungi (basidiomycetes) (Burlat et al., 1997; Hammel, 1997; Tuomela et al., 2000). As most white-rot fungi do not survive the thermophilic phase of composting, these cannot play any significant role in lignin degradation during composting. On the other hand, deuteromycetes and other microfungi, which in contrast to basidiomycetes are present in compost, could be involved in the conversion of lignin in such environments (Kluczek-Turpeinen et al., 2003). Kluczek-Turpeinen et al., 2003 found that the laccases of deuteromycetous fungi seemed to be the key enzymes responsible for the low but steady mineralisation of lignin in compost based on municipal wastes. In some cases, bacterial cells are also seen to be attached to the lignified cell walls, indicating their involvement in degradation (Davis et al., 1992). This was observed in the xylem vessels of wheat straw in these experiments with bacterial colonies on the lignified secondary walls (Fig. 4c). Thus, lignin degradation is seen in compost but is regulated by temperature, the original lignin content and the thickness of the material (Tuomela et al., 2000).

Especially the initial lignin content and the anatomical arrangement of the tissue were expected to influence the degradation in these experiments. In the wheat straw with higher nutrient content and the lignified tissues concentrated in the vascular bundles probably more points of entrance to nonlignified tissues were available for the microorganisms as well as an increased competition due to the higher nutrient levels. Hence, no lignin degradation was seen as the slower growing less nutrient demanding lignin degraders supposedly were out competed. In the hemp and Mischanthus straw the very low levels of nutrients within the tissues probably improved the competition capability of the lignin degraders as these can be inhibited by high nutrient contents (Fog, 1988). This could explain the small decline in the lignin content. The apparent decrease in lignin content in the hemp could also be explained by the way the tissue is broken down. As the hemp straw is degraded into small more flexible structures this increases the accessibility of the microorganisms and thereby enabling lignin degradation.

4.3. Microbiology

The visual measurements were not conducted with sufficient resolution to quantitatively document the changes in dominating microorganisms during the initial phase of composting. However, there was clearly a tendency to lower bacterial and fungal species diversity after one week of composting compared to longer composting time. More fungal species were observed after eight weeks. These results are consistent with the findings of Ryckeboer et al. (2003), showing that species diversity decreased during the thermophilic phase of composting and increased again during the rest of the composting process. The highest number of isolated species was detected during the maturation phase (Ryckeboer et al., 2003). Klamer and Baath (1998) estimated the changes in the relative amount of fungal and bacterial biomass during composting of Mischanthus straw and pig slurry. During the heating phase the fungal/bacterial ratio decreased very quickly reaching a minimum after 6 days, in the following mesophilic phase this ratio increased again but never to the same degree as from the start (Klamer and Baath, 1998). In these experiments we did not analyse the materials until after the thermophilic phase but we observed a similar increase in the fungal biomass during the eight weeks of composting. Thus, the qualitative results of these experiments support visually the molecular findings in other studies.

4.4. Anatomy

The actual degradation of different tissues during decomposition in compost has not been a subject of much research. Generally, composting has been used as a means of mass reduction with focus on what is lost more than what remains. However, microscopic studies of degradation of plant material have been conducted in research considering the digestion of plant parts by rumen microflora (Chesson, 1993; Chesson et al., 1997). The composting process described here is physiologically distinct in that the process is aerobic, whereas digestion by the rumen microflora is an anaerobic process. Composting also differ in that an extremely wide range of microbial organisms comprising mesophilic and thermophilic bacteria, fungi and actinomycetes is present, whereas rumen degradation is based only on a mesophilic microbial flora (Atkey and Wood, 1983). Still, comparisons between the two degradation processes can be made. The rumination of the ruminant animals confirm the importance of the structural properties of plant materials for degradation, as the continuous chewing breaks down the overall structure and increases the points of entry for the rumen microflora, thereby enabling further digestion.

In the wheat straw the nutrient rich tissues, the phloem and the photosynthetic tissues, were degraded rapidly. Access was presumably gained through the cut ends, and as just a small amount of the tissue was lignified this did not present a barrier towards degradation. In the Mischanthus straw less degradation was seen and only the exposed surfaces showed signs of degradation, this probably could be explained by the anatomical arrangement of the tissue and the degree of lignification. The prime limitation to rapid fiber digestion of plant material by rumen microorganisms is believed to be the presence of lignin throughout the walls of the thick-walled cells. However, Wilson and Mertens (1995) showed that in grasses, when microbes have ready access to the surface of the cell walls, then digestion of most of the secondary wall is not prevented by lignification. This was shown in slide-digestion studies with thin slices (<100µm) or when the anatomical structure of lignified tissues was destroyed by isolating and grinding specific cells (Wilson and Mertens, 1995). Previous studies also showed that decomposition of plant residues is size dependent. When the plant material was ground the turnover rate increased, as cells were more readily available to microorganisms (Bremer et al., 1991). Thus, cells throughout the stem can be lignified but are degradable as decomposition is mainly limited by anatomical structure and not by lignification itself (Wilson and Hatfield, 1997).

When free polysaccharides in the cell walls are broken down, a layer of lignified material is left behind inhibiting the further breakdown. Some lignified parts are also released from the cell walls when the surrounding polysaccharides are decomposed (Chesson et al., 1997). Due to the short retention time in the rumen these lignified complexes will not be degraded further, whereas in a compost environment with longer retention time degradation is more likely to occur. Accordingly, the anatomical architecture of the thick-walled lignified fibers of grasses may be as significant a limitation to digestion of secondary walls as is chemical composition. Consequently, due to the large extent of lignification in the Mischanthus straw, degradation of more readily degradable tissues within these straw pieces was inhibited and decomposition occurred more slowly.

In the hemp straw some of the most readily available tissues were already degraded during the retting process in the field. However, of the material that was recoverable from the field we could observe a number of processes during the composting: the degradation of parenchyma cells, primary walls and cellulose layers supporting the secondary wall reinforcements (Fig. 5b and 5c).

4.4. Structure

Compared to wheat straw, hemp and Mischanthus straw are more stable and both could be used as possible peat substitutes. However, the SEM studies revealed large differences in the remaining tissue after decomposition. Dicotyledonous and monocotyledonous species differ in their degradability, which can be explained by anatomical differences (Wilson and Hatfield, 1997). Many dicotyledonous species have a highly lignified xylem ring in the stem, which is not readily degradable, whereas the remaining tissues at the centre and in the periphery of the stem are easily degraded. Monocotyledonous species on the other hand, have their vascular bundles distributed throughout the entire stem and the parenchyma cells contribute significantly to the digestibility problems of the stem; firstly because of the volume they occupy and secondly, because they can develop a thick secondary wall that can lignify (Wilson, 1993). This may have important implications when considering their use as bioresources.

When degraded, the Mischanthus straw breaks up in more rigid pieces than the hemp straw due to the anatomical arrangement of the tissue (Fig. 3a, d, e). The extensive lignification reduces the breakdown and when used as a growing medium it consists of a large amount of defined separate particles. In contrast, when the hemp was degraded it revealed a more flexible structure releasing the lignified secondary helical reinforcements of the xylem vessels. If particles are too large, pore sizes as well will be large leading to fast drainage of the medium. On the other hand, if particles are too small then pore sizes will be so small that the water will be bound tightly and be unavailable for plant roots. The helical reinforcements of hemp having a diameter around 40µm are of such a size that they will be able to connect larger particles creating pore sizes in a relevant range for water retention, that is pore sizes of 30-300µm (Payne, 1988). Thus, we expect that these reinforcements in combination with the external bast fibres of hemp will increase the contact between the particles in the compost and enhance the capillarity and thus the water retention capacity when used as a growing medium. However, the breakdown of hemp straw into small and flexible structures also increases the accessibility of the microorganisms. This enhances further degradation and reduces the stability of the material.

5. Conclusions

The results show that decomposition of straw material during composting is not only dependent on the content of recalcitrant chemical compounds and nutrients but more importantly on the anatomical arrangement of the tissue, as penetration of microorganisms to cells within the straw pieces was impeded by the anatomy of the straw. Additionally, the SEM studies revealed the differences in degradation between species and visualized the structure of the remaining tissues, indicating that hemp might be a good structural element in compost used as a growing medium.

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Appendix III

Long-term stability and mineralisation rate of compost is influenced by timing of nutrient application during composting of plant residues.

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To be submitted to Compost Science and Utilization

Long-term stability and mineralisation rate of compost is influenced by timing of nutrient application during composting of plant residues.

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Abstract

The long-term nitrogen mineralisation and stability of compost based on easily available plant residues was examined. Four different composts were prepared from wheat straw and clover-grass hay, one treatment receiving all material initially, two treatments where addition of 75% of the clover-grass was postponed for 3 respectively 8 weeks and one treatment receiving only 25% of the clover-grass added to the other treatments. Mineralisation and stability of the compost was followed for 24 weeks in leaching tubes, leached regularly by 0.01 M $CaCl_2$. Postponing the addition of the clover-grass hay for 3 weeks during composting had a significant effect on the N mineralisation rate leading to more mineralised N, whereas postponing the addition for 8 weeks showed the same mineralisation pattern, as if all material was added initially. When only 25% of the clover-grass hay was added decomposition was so N limited that almost no net mineralisation occurred. Postponing the addition for 3 weeks also increased the stability of the compost during the initial 14 weeks of incubation; however, after 24 weeks of incubation losses were similar to losses in the treatment receiving all the material from the start. Thus, the postponed addition was shown to alter the mineralisation pattern and stability of the compost in a long-term scale.

Introduction

Organic production of herbs, ornamentals and greenhouse vegetables has developed during the last decades. Growing media for this production are generally based on peat or mixtures of peat and composted material such as animal manure (Eklind et al., 2001). However, as there is increasing focus on exploitation of the peat bogs, and organic animal manure can be sparse a more appropriate growing medium might be produced by composting plant residues. When using compost as growing medium for greenhouse production, the quality requirements are high (Ozores-Hampton et al., 1999).

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One main requirement is high nutrient content. High nutrient availability will in general support microbial growth resulting in a higher degree of degradation (Eiland et al., 2001). Nutritional quality of the compost is dependent on various parameters. Altering the initial C/N ratio or the type of material will influence the mineralisation rate and thus the availability of nutrients (Eiland et al., 2001). Affecting process parameters will lead to changes in microbial communities, which might result in an altered mineralisation pattern (Smårs et al., 2002). Alternatively, recent findings showed that the N mineralisation rate could be managed by postponing the addition of some of the nutrient rich material (Dresbøll and Thorup-Kristensen, submitted).

Splitting the addition of the nutrient rich material at the initiation of a composting process based on wheat straw as a structural element and clover-grass hay as nutritional component was shown to alter the mineralisation rate, generating twice as high mineralised nitrogen levels after 8 weeks of composting (Dresbøll and Thorup-Kristensen, submitted). This could probably be explained by an alteration in the microbial communities during composting. A limited amount of N may be required during decomposition since much C from plant residues such as straw materials is only slowly available to microorganisms. In some cases recycling of N from the microbial biomass may be adequate to meet the N requirements (Bremer et al., 1991). Additionally, microorganisms, especially fungi, have a considerable capacity to adapt to N deficient conditions and a surplus of N initially might then result in increased gross immobilisation, probably due to changes in microbial communities. It has been suggested that there is a high N demand during the first stages of decomposition when soluble and easily degradable C compounds are mineralised while the N demand is lower when the more recalcitrant C compounds are decomposed (Recous et al., 1995). Thus, addition of sufficient N to initiate the decomposition, combined with delayed addition of nutrient rich material when more recalcitrant compounds are degraded in a less N demanding process, could lead to less immobilisation and consequently more mineralised nitrogen (Dresbøll and Thorup-Kristensen, submitted).

Another main requirement of compost used as a growing medium for containerised plants is stability. Stability is mainly dependent on the choice of material but will also be influenced by the nutrient content. Various stability and maturity parameters have been suggested and distinction between the two measurements can be difficult. Maturity is associated with plant growth potential and phytotoxicity whereas stability is associated with the degree of microbial activity (Bernal et al., 1998; Eggen and Vethe, 2001). As phytotoxic compounds are produced by microorganisms in unstable compost the two terms are closely related. Amongst others, a low C/N ratio and a high NO_3^-/NH_4^+ ratio are often used as maturity parameters (Bernal et al., 1998) whereas stability in general is determined by respiration rate or heat development. When using compost as a growing medium for containerised plants, stability is furthermore critical as large volume losses should be avoided (Jensen et al., 2001; Gruda and Schnitzler, 2004). The structure of the material after composting will also affect the volume losses when used as growing medium, as the particle size distribution influences the way the media is compressed by gravity (Gruda and Schnitzler, 2004).

This study is a natural extension of the studies by Dresbøll and Thorup-Kristensen (submitted) and is based on the compost produced with postponed addition of part of the nutrient rich material. The objective was to examine the nutritional quality and stability of plant-based compost to be used as growing medium. It was hypothesised that the postponed addition of part of the nutrient rich material would affect the N mineralisation leading to more available N. The postponed addition was expected to influence the decomposition of recalcitrant compounds in the compost and thereby alter the stability as growing medium. In a semi-natural environment we examined the changes in C/N, mineralised N, mass loss and volume loss during a long-term incubation experiment. Growth conditions were simulated by keeping the compost in tubes and leaching these regularly.

Methods

Experimental design and materials - Composting

Compost for the leaching tube experiments was made of wheat straw as structural component and clover-grass hay as a nutrient rich component. Details on the materials and composting processes are reported in Dresbøll and Thorup-Kristensen (submitted). In short, three different treatments with three replicates were compared. The first treatment was a mixture of wheat straw and clover-grass hay giving a C/N-ratio of 33 (full-mix treatment). The second treatment received only 25% of the clover-grass hay initially resulting in a C/N ratio of 54. Three weeks later the remaining 75% of the clover-grass hay was added (short-delay treatment). The third treatment received only 25% of the clover-grass hay; no additional clover-grass hay was added during composting in this treatment (25%-treatment).

Experimental design and materials - Leaching tubes

During the leaching tube experiment four different compost types were compared. The compost from the three treatments during the composting period was used. Additionally, one treatment (long-delay treatment) was made by applying the remaining clover-grass hay to the 25%-treatment from the composting experiment, just before incubation in the leaching tubes; accordingly this treatment received the same amount of material as the full-mix and short-delay treatments during composting.

The leaching tubes were constructed of 25 cm long perspex glass tubes with an inner diameter of 7 cm, containing 850 cm^3 of compost. The bottom of the tubes was covered with a nylon mesh, whereas the upper end could be sealed with a plastic lid. During the experiment the tubes were kept in a dark room with controlled climate having a temperature of 20°C.

Sampling and volume measurements

During the initial six weeks the tubes were leached once a week. Hereafter, leaching intensity was reduced and during the following 2.5 month leaching was conducted every two weeks and finally once a month for the last two months. When leached, the plastic lids were mounted on the tubes and they were turned. Leaching solution (0.01 M $CaCl_2$) was added until the compost was completely covered, left for 45 minutes, turned again and drained through the nylon mesh for 30 minutes. 4 M KCl was added to the leachate to reach a final concentration of 1 M KCl, samples was filtered and stored frozen until analysis for NO_3^- and

NH_4^+. During the leaching tube experiment the changes in structure and stability of the compost was measured by the reduction in compost column height in the tubes and the degree of volume loss was determined.

Physical and chemical analysis

As the leachate extracts were strongly coloured by organic compounds, they were cleared by shaking the extract with active carbon for 15 minutes and filtered (0.45µm pore size) to avoid interference when measured spectrophotometrically. The analysis for NH_4^+ and NO_3^- content was conducted by standard colorimetric methods using flow-injection analysis (FIA) (Keeney and Nelson, 1982).

At the initiation of the experiment, estimates of dry weight and initial C and N content was determined for the four treatments and after 24 weeks the remaining compost was subsequently dried and analysed for C and N content. Total C and N content was determined by dry combustion of dried and finely ground samples with an automated N-C analyser interfaced with an isotope mass spectrometer (Carlo Erba, EA 1108). Samples were acidified by pouring 60 ml 0.1M HCl over the entire sample before drying to avoid NH_4^+ losses.

Hot water solubles, ADF, cellulose and lignin content was measured by C-fractionation in a fiber analyser (ANKOM^{220} Fiber Analyzer) at initiation of incubation and after the 24 weeks of incubation.

Statistics

The results were calculated as an average of three replicates of each treatment in the composting experiment and of eight replicates during the leaching tube incubation. Data were analysed with the GLM procedure of the SAS statistical package (SAS Institute Inc., Cary, NC, USA)

Results

Composting experiment

The main results of the composting experiment are presented in the paper by Dresbøll and Thorup-Kristensen (submitted). In short, initial nitrogen levels were too low to see the effect of the postponed addition on the mineralisation rate. A small amount of NH_4^+ was detected whereas no net mineralisation of NO_3^- was observed. Thus, after 8 weeks of composting no significant differences in mineralisation was observed between the three treatments. When the compost was used in the leaching tube experiment it had not reached a level of net mineralisation yet, which affected the stability of the compost.

C-to-N ratio and mass losses

At the initiation of the leaching tube incubation the C/N ratio were just below 20 for the full-mix and short-delay treatments whereas the other treatments differed significantly with initial C/N ratios of 39 and 30 for the 25% and the long-delay treatments respectively (Fig 1a).

During the incubation period the C/N ratio decreased in all treatments, in the full-mix, short-delay and long-delay treatments a final C/N ratio of 13-15 was observed with no significant differences between the treatments. In the 25% treatment the C/N ratio declined to 31 during the incubation time. Final C/N ratios were determined on the basis of the N left in the compost after leaching.

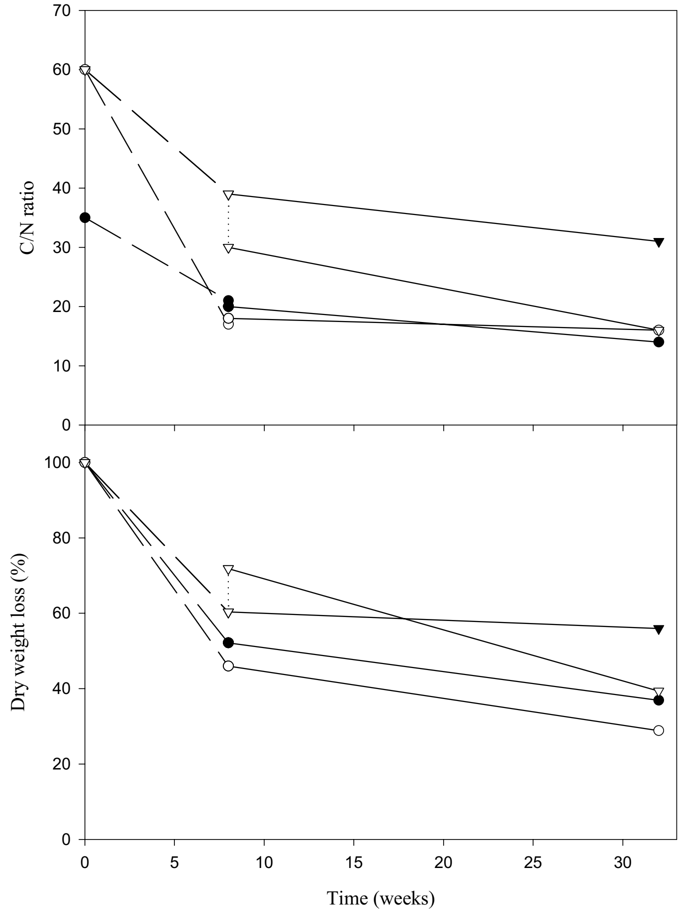

Fig. 1. a) Changes in C/N ratio during composting (dashed line) and subsequent incubation in leaching tubes (solid line). The dotted line indicate the decrease in C/N due to the supplementary addition of clover-grass hay before incubation in the 8-weeks-delay treatment b) Total loss of initial dry weight during composting (dashed line) and incubation (solid line). The dotted line represents the increase in material dry weight when clover-grass hay was added before incubation in the 8-weeks-delay treatment. The full-mix treatment (●), the short-delay treatment (○), the 25%-treatment (▼), the long-delay treatment (▽).

Mass losses during the leaching tube incubation varied between the four treatments. The highest mass loss of 45% of the material in the tubes was seen in the long-delay treatment

receiving the additional clover-grass hay just before incubation was initiated. The short-delay treatment with addition of the remaining clover-grass hay postponed for three weeks, and the full-mix treatment having all the material from the start, followed with losses of 37% respectively 29%. The 25%-treatment being different from the other treatments, as no supplementary material was added, lost only 7% of the mass during the incubation period (data not shown). Differences between all treatments were significant ($p<0.01$).
Calculated as the total loss of compost from the initiation of the composting process to the end of the leaching tube incubation, the short-delay treatment had a higher total loss than the long-delay treatment of 73% compared to 63% (Fig.1b). These treatments were followed yet again by the full-mix treatment with losses of 56% and the 25%-treatment with a total loss of 36%. Total losses of the four treatments differed significantly ($p<0.01$).

Nitrogen mineralisation

Nitrogen mineralisation was significantly altered by the short-delay addition of part of the clover-grass compared to the other treatments (Fig 2). After 16 weeks of incubation and forward, the accumulated mineralised N was significantly higher in the short-delay treatment. The actual peak in mineralised N was observed after 12 and 14 weeks being significantly higher than the other treatments. During the first 10 weeks of incubation in the leaching tubes the mineralisation in all treatments were very low and no clear differences in the accumulated mineralised N content was observed. The accumulated mineralised N content in the 25%-treatment was significantly lower than the other treatments for the rest of the incubation period. No significant differences were observed between the accumulated mineralised N content of the full-mix and the long-delay treatments at any time during the experiment.

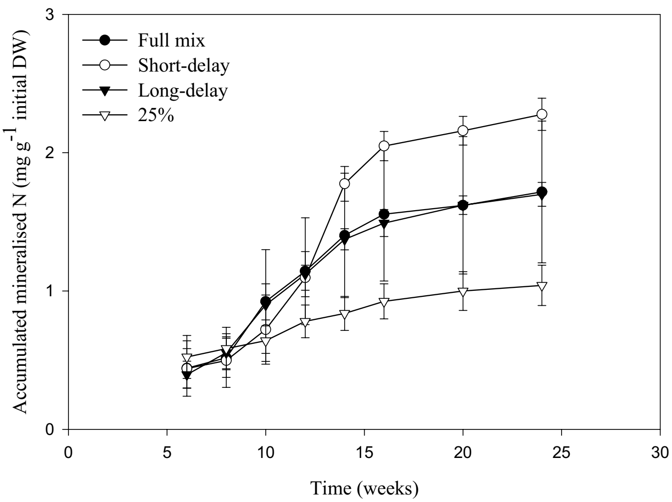

Fig. 2. Accumulated mineralised nitrogen (g g^{-1} initial DW) during incubation in leaching tubes. Results are means of three replicates and bars show standard deviation.

Fibre content

The sum of hot water solubles (HWS) and acid detergent fibres (ADF) made up approximately 50% of the material initially in all four treatments (Table 1). After the incubation time the long-delay treatment had a significant higher loss than the other materials whereas the sum of HWS and ADF loss were significantly lower in the 25%-treatment. No significant difference in loss was observed between the full-mix and the short-delay treatments.

There were no significant differences between the initial cellulose content in the four different treatments, all having cellulose contents just around 30 % (Table 1). The lowest cellulose decomposition during the leaching tube incubation was seen in the 25%-treatment with a loss of 25.1% whereas losses of 58-63% were observed in the remaining treatments.

No significant differences were observed in the initial lignin content in the different treatments either (Table 1). However, after the 24 weeks of incubation the loss of lignin in the short-delay treatment was significantly ($p<0.05$), higher than the remaining treatments, where no difference was observed.

Table 1. The initial and final concentration of hot water solubles and acid detergent fibres, cellulose and lignin in the four compost treatments and percentage mass loss of each fraction after incubation. Results are means of three replicates and standard deviation are shown in brackets.

Treatment	Hot water solubles + ADF			Cellulose			Lignin		
	%								
	Initial conc.	Final conc.	Loss	Initial conc.	Final conc.	Loss	Initial content	Final conc.	Loss
Full-mix	49.7 (1.8)	49.1 (2.8)	30.2	30.0 (2.4)	17.7 (1.2)	58.2	16.3 (3.4)	25.9 (2.8)	-12.3
Short-delay	52.0 (1.6)	56.0 (2.4)	32.3	26.4 (1.9)	16.9 (0.2)	59.9	18.0 (1.2)	21.5 (1.8)	25.2
Long-delay	55.2 (1.7)	48.3 (2.4)	52.1	28.4 (2.0)	19.0 (1.9)	63.4	14.2 (3.2)	25.4 (0.6)	1.6
25%	49.5 (1.0)	48.7 (1.0)	8.8	29.0 (0.5)	23.5 (0.9)	25.1	18.7 (1.5)	22.9 (1.1)	-13.7

Total mass losses of the composts were 29% (full-mix), 37% (short-delay), 45% (long-delay) and 7% (25%).

Stability

Stability of the compost was determined as the degree of volume loss during the incubation time and was measured as the height of the compost in the leaching tubes during the last three months of incubation (Fig. 4). In the long-delay treatment the highest degree of volume loss was seen throughout the entire experiment ($p<0.05$). The full-mix treatment had a tendency to a steeper decrease in compost height during the first 12 weeks than the short-delay treatment although no significant difference in the degree of volume loss was observed in these treatments. After 14 weeks a steep decrease in compost height was observed in the short-delay treatment coinciding with increased mineralisation. The 25%-treatment had a significantly lower degree of volume loss than the other treatments and lost less than 20% of the initial height during the entire period.

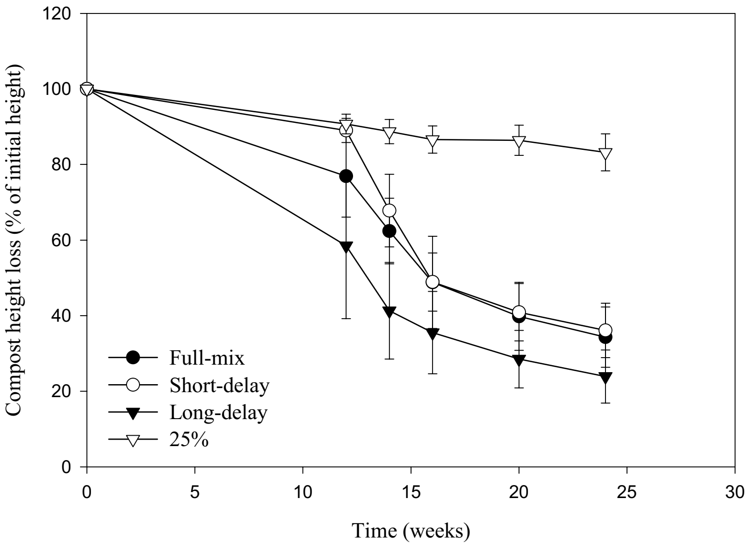

Fig. 3. Volume loss in the four treatments expressed as percentage compost column height loss. Results are means of eight replicates and bars show standard deviation.

Discussion

Mineralisation

Postponing the addition of some of the nutrient rich material in the short-delay treatment during the composting process did not show the expected effect on the mineralisation rate as no net mineralisation was observed. Despite that, large differences in the mineralisation pattern were observed between the four treatments during the subsequent incubation. The short-delay treatment had a higher content of accumulated mineralised N, whereas postponing the addition for 8 weeks did not alter the mineralisation pattern, compared to the full-mix treatment receiving all the material from the start. However, even though net mineralisation patterns are comparable, this does not confirm that the processes behind are similar as the mineralisation and immobilisation processes take place simultaneously (Amlinger et al., 2003) and has not been examined here. Although delayed due to the low nutrient content, the mineralisation pattern in the short-delay treatment corresponded to the findings of Dresbøll and Thorup-Kristensen (submitted), that postponed addition of some of the nutrient rich material can increase the mineralisation of nitrogen. This has been explained by the changes in microflora occurring when N accessibility is altered.

Despite the similarities in postponing the addition of some of the nutrient rich material in the short-delay and the long-delay treatments, the delay affected the two treatments very differently. In the short-delay treatment the extra addition resulted in an additional temperature increase to thermophilic conditions, whereas the addition in the long-delay treatment did not affect the temperature due to the setup, and thus the following degradation could not be ascribed to composting but to decomposition at a constant low temperature of 20°C. Composting is generally defined as an aerobic degradation of organic matter under development of heat. Temperature is a significant factor in determining the relative advantage of some population over another and is thus, the dominant physiochemical parameter controlling microbial activity during composting (Ishii and Takii, 2003). Hence, the main part of the decomposition in the long-delay treatment has been dominated by different microorganisms than in the short-delay treatment, where part of the decomposition was controlled by thermophilic microorganisms. This might be part of the explanation for the different patterns observed.

The C/N ratio decreased only to 13-15 during incubation, in the full-mix, short-delay and long-delay treatments, as N continuously was removed by leaching. If the leached accumulated N was included in the determination of final C/N ratio, estimates of a C/N ratio of 9-10 was obtained. During the incubation period, the most profound changes in C/N ratio (Fig. 1a) and mass (Fig. 1b) occurred in the long-delay treatment, as almost no decomposition had occurred before the incubation period. Thus, when clover-grass hay was added to the compost just before incubation in the tubes microbial activity increased and further decomposition occurred. Even in the treatments which received the nutrient rich material during the previous composting process high mass losses were observed. The losses during the leaching tube incubation confirmed that decomposition during the 8 weeks of composting was nutrient limited and the decomposition rate thus inhibited. However, some decomposition had occurred, confirming that despite the N deficiency during decomposition, C mineralisation occurred although the decomposition rate was much lower (Recous et al., 1995). The nitrogen limitation in the 25%-treatment was clear even after the incubation period, as weight losses during the period were low and the C/N ratio still above 30.

During a composting process the highest mass losses occur during the initial mesophilic phase and the shift from the mesophilic to the thermophilic phase as these are the periods of highest microbial activity due to the availability of carbohydrates (Bernal et al., 1998; Klamer and Baath, 1998). Initially, easily available carbohydrates are decomposed followed by breakdown of proteins, lipids, and complex carbohydrates which is accelerated by the high temperatures of the thermophilic phase, hence leading to high mass losses. During composting and incubation the total mass losses were largest in the short-delay treatment which could be explained by the extra rise in temperature when the supplementary clover-grass hay was added reflecting the increased microbial activity. As the supplementary clover-grass hay was added right before initiation of the incubation period in the long-delay treatment no similar temperature increment was observed in this treatment due to the small amount of material in the tubes and no insulation.

Stability

Despite having received the same amount of material although at different times, the full-mix, the short-delay and the long-delay treatments differed in the way the material were lost during the incubation time. As the long-delay treatment received the clover-grass hay right before incubation, a higher loss was expected. During the previous composting process the compost of the 25% and the long-delay treatments only lost a small amount of the mass as degradation was nutrient limited. This tendency continued in the incubation experiment for the 25%-treatment whereas the long-delay treatment had high losses after the application of the clover-grass hay. Losses during the incubation period could be ascribed to CO_2 release and inorganic N in the leachate. In addition some organic N could have been lost in the leachate, as small particles of organic matter.

The degree of volume loss in the full-mix, the short-delay and the long-delay treatments revealed visually what was measured in mass loss and initial C/N ratio that is the instability of the composts. Used as a growing medium for containerised plants this would not be satisfactory (Gruda and Schnitzler, 2004) as the growing medium would almost have disappeared during the production time. However, the presence of roots in the growing medium would have stabilised it somewhat more than the growing medium alone. In soil, roots and microorganisms compete for nutrients (Jingguo and Bakken, 1997), why the presence of roots could have inhibited further C mineralisation in the compost as plant roots would have inhibited microbial decomposition by competing for the available nutrients. In general, roots have the competitive advantage compared to microorganisms as roots grow and is distributed in the medium and thereby can exploit a larger volume for available N. Furthermore, the mere presence of roots would also stabilise the media and compaction would be reduced. Still, some volume loss is expected ranging from 15-50 % when using compost based growing media for plants with long production time, although compression of the growing medium in the containers before planting could reduce the volume losses (Jensen et al., 2001; Gruda and Schnitzler, 2004).

The highest volume and mass losses were found in the long-delay treatment followed by the full-mix and short-delay treatments. The 25%-treatment had significantly lower volume and mass losses. Interestingly, the losses of volume and the losses of mass differed considerably in all four treatments. In the long-delay treatment a volume loss of 75% corresponded to a mass loss of 45%, revealing the structural breakdown of the material. Although only half of the material was lost due to microbial respiration the structure of the material was altered so much during decomposition that compaction increased due to smaller particle sizes and thereby less air filled porosity. Such a volume loss caused by reduction in particle size and compression due to gravity results in a reduction of macropores together with an increase in micropores (Gruda and Schnitzler, 2004). This will lead to higher water retention but less aeration. As the air-filled porosity decrease with time and aeration properties cannot easily be changed during plant growth, high initial aeration levels are essential (Caron and Nkongolo, 1999).

Fibre content

No significant differences were found between the hot water solubles and ADF, cellulose and lignin content of the four treatments at the start of the incubation period indicating a similar degradation pattern independent of the clover-grass amendment. Lignin degradation did not occur during incubation with exception of the short-delay treatment where a decrease in lignin content was observed. This might be explained by the altered microflora caused by the 3 weeks delay in addition of the nutrient rich material allowing the presence of slow growing lignin degraders.

Conclusion

Postponed addition of some of the nutrient rich material during composting was shown to affect the subsequent mineralisation pattern in the compost when followed for 24 weeks in leaching tubes. This could probably be explained by changes in the microbial communities. However, the exact mechanisms leading to the altered mineralisation pattern is yet unknown and more knowledge of the dynamics of microbial communities are needed. Still, this is a simple way of inducing more available N in compost used as organic growing medium which can be of great importance for plant growth. A disadvantage of the postponed addition is the higher mass loss during composting. However, the short-delay treatment with the supplementary addition postponed for 3 weeks remained stable for a longer period than the other treatments and might be a functional improvement as growing medium for plants with a shorter growth period.

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Appendix IV

Structural differences in wheat (*Triticum aestivum*), hemp (*Cannabis sativa*) and Mischanthus (*Mischanthus ogiformis*) affect the quality and stability of compost as growing medium

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Structural differences in wheat (*Triticum aestivum*), hemp (*Cannabis sativa*) and *Mischanthus* (*Mischanthus ogiformis*) affect the quality and stability of compost as growing medium

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Abstract

Physical properties as well as process parameters were examined in three different composts based on plant residues. The wheat compost was a mixture of clover-grass and wheat straw in a ratio of 3:5, the *Mischanthus* compost was composed of the same materials and contained *Mischanthus* straw in addition in a ratio of 3:2.5:2, and the hemp compost was based on clover-grass, wheat and hemp straw also in a ratio of 3:2.5:2. The wheat and *Mischanthus* composts both had an initial C/N ratio of 26 and process parameters such as nitrogen losses and mineralisation pattern did not differ between the two compost types. The hemp compost had a much lower initial C/N ratio of 16 as the hemp straw was harvested fresh, resulting in higher losses and more mineralised nitrogen during composting. Particle size distribution was determined by image analysis of scanned samples. No differences were observed between the percentages of particles in the selected length intervals, however the largest number of particles was observed in the *Mischanthus* compost. Particles in the hemp compost had more uneven surfaces than the other composts and had the highest water retention capacity. Thus, the geometry and surface characteristics of the particles and the pores created are important in determining suitability of plant based growing media.

Keywords: Compost; Straw; Decomposition; Particle size distribution; Water retention capacity

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1. Introduction

Decomposition of plant tissue is dependent on various factors including temperature, moisture content, oxygen content and residue quality (Fog, 1988; Eklind and Kirchmann, 2000b; Eiland et al., 2001; Smårs et al., 2002). In general, both resource quality and physiochemical parameters influence the composition and activity of the decomposer communities conducting the mineralisation/immobilisation processes of decomposition.

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Thus, when producing plant based compost to be used as growing medium in horticultural productions choice of plant material is a key factor, as root proliferation and development is dependent on the physical structure and stability of the medium. Another important factor affecting the successful use of composted plant residues as growing media is management of the composting processes in order to provide sufficient and balanced nutrient release. Nutrient release can be influenced by initial C/N ratio (Eiland et al., 2001), type of nutrient rich material (Sánchez-Monedero et al., 2001) and time of addition of the nutrient rich material (Dresbøll and Thorup-Kristensen, submitted). Clearly, nutrient availability in composts used as growing media can also be enhanced by extra addition of fertilisers during production.

The physical properties are mainly dependent on the starting material and are difficult to alter during production (Caron and Nkongolo, 1999). An apparent option to improve the structure and stability of compost is to alter the composition of plant material and species, which are composted. Using materials with different contents of lignin and cellulose as well as different morphological properties is expected to influence the structure and stability of the mature compost. Lignin content and the distribution of it in cells can affect decomposition, by acting as a physical or chemical barrier towards decomposition of cellulose and other carbohydrates (Martin et al., 1980; Melillo et al., 1982).

Altering the type and amount of structural material in the compost will influence the physical structure and stability as well as the temperature development, moisture content, oxygen content and hence the decomposer community in the compost (Eklind and Kirchmann, 2000a; Beck-Friis et al., 2003). Thus, it is not possible just to change the physical structure; changing one factor affecting the composting process will affect all the other parameters and result in a compost of different properties. All composting parameters are closely connected so even small changes may affect the microbial communities and thereby the overall compost quality (Leth et al., 2001).

Degradation of plant material is not only dependent on the lignin content but also on the arrangement of the different tissues. Dicotyledons and monocotyledons differ in their degradability, which can be explained by anatomical differences (Wilson and Hatfield, 1997). Many dicotyledons have a highly lignified xylem ring in the stem, which is not readily degradable, whereas the remaining tissues at the centre and in the periphery of the stem are easily degraded. Monocotyledons on the other hand, have their vascular bundles distributed throughout the entire stem and cells throughout the stem can be lignified (Wilson and Hatfield, 1997).

To ensure suitable structure and stability in compost, the lignin content and arrangement of lignified tissues are important as well as aeration and water retention capacities of the compost are key factors in successful plant growth (Allaire et al., 1996; Gruda and Schnitzler, 2004). Particle size distribution and more importantly the geometry of the particles and surface characteristics are parameters influencing the physical properties of a growing medium. Plants having flexible structures such as helical secondary wall reinforcements or extraxylary fibres such as the external bast fibres of hemp might then be more suitable as a structural component. Many dicotyledons contain more of these cells than monocotyledons as the xylary ring make up a large part of the stem tissue (Wilson and Hatfield, 1997). The flexible structures enhance the connection between particles, thus creating continuous pores and improving capillarity.

The aim of this study was to compare the physical properties of compost based on wheat straw and clover-grass hay with compost based partly on the same materials but with additional amendments of two different structural materials, straw of the monocotyledonous *Mischanthus* and straw of the dicotyledonous hemp. It was hypothesised that the hemp-based compost would have superior physical properties when compared to wheat and *Mischanthus* composts, as hemp was expected to degrade into more flexible structures. The effect of the different amendments on compost quality, defined as nutritional value, particle size distribution, aeration and water retention capacity was observed as these are all important parameters when compost is used as a horticultural growing medium.

2. Materials and Methods

2.1. Compost

Three different types of compost, based on clover-grass hay as a nutrient rich component and wheat, *Mischanthus* or hemp straw as structural components, were compared. The wheat compost was a mixture of clover-grass hay (30 kg) and wheat straw (50 kg) giving an initial C/N ratio of 26. The *Mischanthus* and hemp composts contained the same materials but half of the wheat straw was replaced by 20 kg *Mischanthus* respectively hemp straw. The C/N ratio of the *Mischanthus* compost was 26 whereas the C/N ratio in the hemp compost was only 16, as the hemp material used were harvested fresh and had a high N content. The main objective was to examine physical properties of the composts, and the amount of the different materials added were prioritised over C and N content, when determining the proportions of the materials. Shredded (<20 mm length), oven dried clover-grass hay was used, whereas the straw materials were air dried and shredded to pieces of <50 mm length. The plant materials were mixed and watered to a water content of 60-65%, and placed in 800 L wooden compost boxes measuring 0.7 x 1.0 x 1.2m (height x width x length). Additional water was added whenever necessary during the composting time to keep the water contents above 50%. Heat loss was minimised by insulation with glass-wool mats and the boxes were passively aerated by heat convection. After 3 weeks of composting the compost was turned, and the total composting time was 10 weeks.

2.2. Sampling

Samples were collected at the initiation of the composting process and after 3, 7½ and 10 weeks of composting. At initiation, after 3 weeks and after 10 weeks samples were collected after the compost was mixed thoroughly, approximately 250 g compost was collected and subsamples for further analysis were obtained from this. After 7½ weeks 5 samples of approximately 50 g was collected randomly in the compost pile and pooled. Subsamples of the pooled mass were collected for analysis. Temperature was measured continuously in the centre of the composting boxes using standard acid proof stainless steel Pt-100 probes connected to a data logger (Datataker DT500).

2.3. Weight losses

Compost boxes were weighed at initiation of the composting process, when turned after three weeks and finally after 10 weeks and weight losses were determined. In addition, nylon mesh litterbags (mesh size 1x1.2mm) were placed in the compost boxes of the *Mischanthus* and hemp composts. In the *Mischanthus* compost litterbags containing wheat straw (3 g), *mischanthus* straw (3 g) or a mixture of the two straw types (1.5 g of each) was placed whereas the hemp compost had litterbags containing wheat straw, hemp straw or a mixture of these materials in the same amounts as in the *Mischanthus* litterbags. This made it possible to follow the actual weight losses of the structural components and the effect of mixing them.

2.4. Physical and chemical analysis

Water content was determined by weight loss of compost samples, which were oven dried at 80°C for 24 hours. Total N and C were measured by dry combustion of dried and finely ground samples with an automated N-C analyser interfaced with an isotope mass spectrometer (Carlo Erba, EA 1108). Samples were acidified by pouring 60 ml 0.1M HCl over the entire sample before drying to avoid NH_4^+ losses.

The NH_4^+ and NO_3^- content was determined in a 2M KCl extract (compost (20 g fresh weight): solution ratio 1:10) followed by shaking for 45 minutes and centrifugation. The supernatant was filtered through Advantec 6 (Frisenette Aps.) filters and stored frozen. As the extracts were strongly coloured by organic compounds, they were cleared by shaking the extract with active carbon for 15 minutes and filtered (0.45µm pore size) to avoid interference when measured spectrophotometrically. The analysis for NH_4^+ and NO_3^- content was conducted by standard colorimetric methods using flow-injection analysis (FIA) (Keeney and Nelson, 1982).

2.5. Particle size distribution

After the composting period the particle size distribution was measured for the three compost types. Approximately 1g of compost was placed in water for 24 hours, distributed evenly on a perspex tray and scanned on a flat bed scanner. The scanned image was analysed by the software program WinCam (Regent Instruments Inc.) measuring particles at 600 dpi corresponding to a pixel size of 0.042 mm in length. Length, width and amount of particles are given by the software and particle size distribution was determined. An estimate of the particle volume was produced by a simplified assumption that height and width of particles were identical, and was calculated as length*width^2. This is of course a rough estimate, however, volume of particles provide more information on the physical properties than particle area.

2.6. Water retention

Water retention capacity and air-filled porosity was measured on a sandbox. Samples were prepared and measured according to the European standard (PrEN 13041, 1999; EN 13040, 2000). Compost was filled in containers, saturated in water and equilibrated on a sand box at a water tension of 5 kPa. The compost was then transferred into double ring sample cylinders, rewetted and equilibrated at 1 kPa water tension. The rings were separated, and the compost of the upper ring was discarded. Hereafter, the lower ring contained a known volume of compost. Water retention was measured at tensions of 1,2,3,5 and 10 kPa. Samples were then dried at 103°C and water retention and air-filled porosity was determined from the obtained results.

2.7. Statistics

The results were calculated as an average of three replicates of each treatment (five replicates for determination of particle size distribution and water retention) and analysed with the GLM procedure of the SAS statistical package (SAS Institute Inc., Cary, NC, USA)

3. Results and Discussion

3.1. N mineralisation

The N mineralisation was similar for the wheat and *Mischanthus* composts (Fig.1a and 1b) despite the difference in material. In the hemp compost more mineralised N was observed reflecting the higher initial N content and thus much lower C/N ratio. No significant differences was observed between the wheat and *Mischanthus* compost, whereas the hemp compost had significant higher NO_3^- and NH_4^+ contents all through the composting experiment except the NH_4^+ content at initiation.

The high initial NO_3^- content revealed that the hemp was harvested green and fresh. Initially the NO_3^- content of the wheat and *Mischanthus* composts rose, but after 7½ weeks of composting it started to decrease. In the hemp compost, the NO_3^- content fell already after 3 weeks, and the decline was much stronger. A decline in NH_4^+ content is generally seen during composting (Dresbøll and Thorup-Kristensen, submitted) as it is oxidised to NO_3^-. However, a decline in NO_3^- content is probably connected to losses due to denitrification. If anaerobic zones in the compost have been created NO_3^- is used by the microorganisms as oxygen source which results in denitrification and lead to N losses (Sommer, 2001; Sánchez-Monedero et al., 2001). A decline in NO_3^- could also be explained by immobilisation, yet, a significant loss in total N content was observed excluding immobilisation as an explanation. Anaerobic zones are created, especially if the water content is too high (Agnew and Leonard, 2003). However, this could also lead to leaching losses.

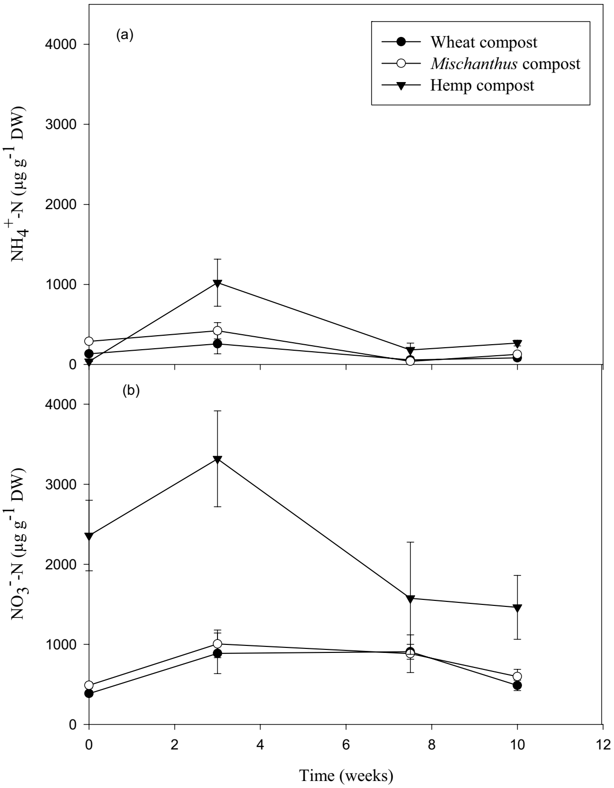

Fig.1. Mineralisation pattern during composting (a) ammonium content and (b) nitrate content. Wheat compost: wheat straw and clover-grass hay (●), *Mischanthus* compost: wheat straw, *Mischanthus* straw and clover-grass hay (○), and hemp compost: wheat straw, hemp straw and clover-grass hay (▼). Results are means of three replicates and bars show the standard deviation.

3.2. Temperature

The temperature in the composts followed the pattern generally observed during composting (Fig 2). An initial fast increase to thermophilic temperatures above 70°C was followed by a decrease to mesophilic temperatures. When the compost was turned after 3 weeks of composting another temperature peak was measured with temperatures above 60°C. The temperature increase is generated by the aerobic metabolism of microorganisms, and is therefore affected by the availability of O_2. When turning the compost to obtain homogeneity of samples after 10 weeks, this resulted in an additional small increase in the temperature of the three types of compost. The reactivation of the degradation process could be explained by the incorporation of material from the exterior into the compost thereby providing fresh substrate for the microbial biomass (Garcia-Gomez et al., 2003). If anaerobic conditions have been present, the turning of the compost and thereby incorporation of oxygen would also lead to an increase in aerobic microbial activity.

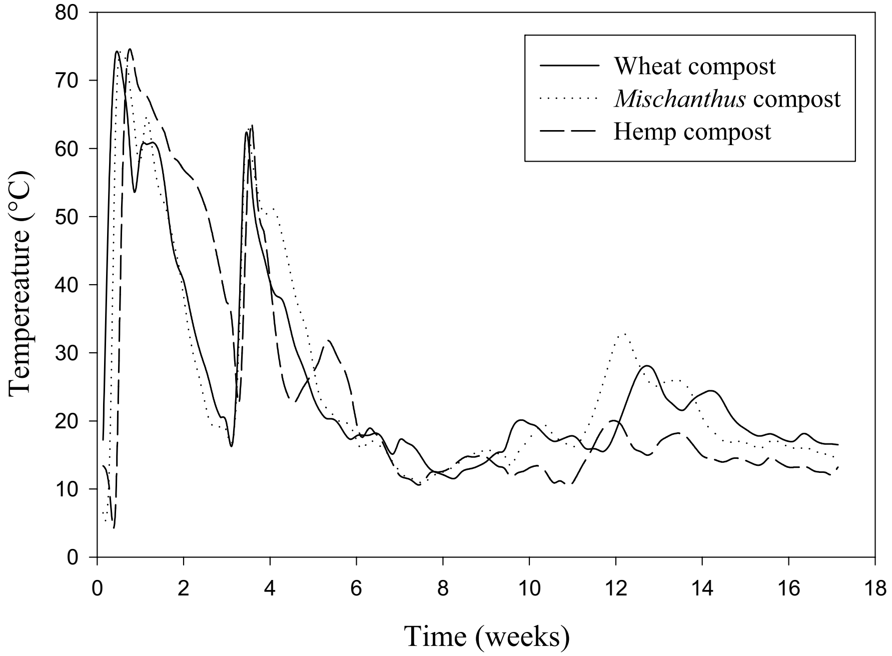

Fig.2. Temperature development during composting. Wheat compost: wheat straw and clover-grass hay (solid line), *Mischanthus* compost: wheat straw, *Mischanthus* straw and clover-grass hay (dotted line), and hemp compost: wheat straw, hemp straw and clover-grass hay (dashed line).

3.3. Weight losses and C/N ratio

No significant differences was detected between the weight loss of the wheat and *Mischanthus* composts, both lost 65% of their dry weight, whereas the hemp compost differed significantly with a loss of almost 80% (Table 1). Similar patterns were observed with the C/N ratio of the material with no differences between the wheat and *Mischanthus* composts both starting with a C/N of 26 declining to 15 after 10 weeks of composting (Table 1). As the hemp straw was fresh newly cut material the N content was much higher than the structural materials of the wheat and *Mischanthus* composts. With the same amount of hemp, as *Mischanthus* straw in the *Mischanthus* compost, the initial C/N ratio was 16 decreasing to 11 after the composting period.

Mass losses during composting of different materials such as manure or household waste are generally found in a wide range from about 40 to 80% (Sommer and Dahl, 1999; Eklind and Kirchmann, 2000a). Mass losses are mainly dependent on composting time and type of material (Eklind and Kirchmann, 2000a). The losses of the wheat and *Mischanthus* composts were comparable to losses previously found in compost based on plant materials (Dresbøll and Thorup-Kristensen, submitted). However, the losses from the hemp compost were unsuitably high, as mass retention is desirable if compost should be used as a growing medium. The fresh hemp material was easily degraded as N was sufficient. Lignin content in hemp has been shown not to change significantly from 30 to 120 days of growth (Kamet et al., 2002). This indicates that lignification itself is not determining the decomposition rate. Hence, the distribution of lignin in young hemp tissue might not impede the access to easily degradable carbohydrates.

A large amount of the total weight loss could also be ascribed to losses of the nutrient rich clover-grass hay (Table 1). In order to follow the actual decomposition of the structural elements, litterbags were placed in the compost piles. The *Mischanthus* straw and the hemp straw alone both differed significantly from any of the other materials, the *Mischanthus* straw with losses of only 17% and hemp straw with the highest losses of 55%. When the *Mischanthus* straw was mixed with wheat straw the losses did not differ significantly from wheat straw alone, likewise the hemp straw mixed with wheat did not differ from the wheat straw alone. In addition, the losses of the wheat straw in the two compost types did not differ significantly from each other. In previous studies, the actual losses of *Mischanthus* and mature retted hemp straw was both found to be 17% after 8 weeks of composting (Dresbøll and Magid, submitted). Thus, the losses of the hemp material used in these studies differed significantly from losses in mature hemp, indicating that the abundance of N increased the degradation.

Table 1. Dry weight loss and C/N ratio after 10 weeks of composting in the wheat compost (wheat/clover-grass), the *Mischanthus* compost (wheat/*Mischanthus*/clover-grass) and the hemp compost (wheat/hemp/clover-grass). In addition, dry weight loss of plant materials in litterbags from the *Mischanthus* and hemp composts are shown. Results are means of three replicates with standard deviation shown in brackets.

Material		**C/N ratio**	**C/N ratio**	**Loss (%)**
			After 10 weeks of composting	
Wheat compost		26	15.2 (1.4)	64.9 (3.6)
Mischanthus compost		26	15.1 (1.1)	65.2 (3.7)
Hemp compost		16	11.0 (0.8)	79.4 (0.5)
Mischanthus compost:	Wheat			37.1 (5.8)
	Mischanthus			16.8 (8.6)
	Wheat/*Mischanthus*			28.4 (3.7)
Hemp compost:	Wheat			39.0 (4.5)
	Hemp			55.5 (2.1)
	Wheat/hemp			41.8 (4.0)

3.4. Particle size distribution

The particle size distribution, determined as percentage of particles in each length interval, did not differ between the three compost types (Fig. 3b). However, the total number of particles in the interval 0.0-0.1 was significantly lower in the hemp compost and higher in the *Mischanthus* compost than in the wheat compost (Fig. 3a). The percentage of particles in each length interval is the most accurate measurement. Samples were taken from fresh compost material and the total number of particles could reflect differences in water contents of the composts rather than an actual difference in amount of particles. However, the large differences found in the 0.0-0.1 interval are not believed to be due to differences in water content alone. The small particles (< 1 mm) comprised the largest number of the particles. However, they comprised just a negligible part of the estimated total volume of particles (Fig. 3c). The relatively few large particles were responsible for up to 40% of the total volume. An ideal particle size distribution is difficult to define. Composted plant material used as growing media should contain particles of different sizes. Ideally, the medium should be composed of particles that creates pores in the range of 30-300μm in diameter (Payne, 1988) as these allow water retention and allow water uptake by roots at the water content range relevant for containerised plants. Pores are created not only between particles but also within the particles and the pore geometry will be dependent on surface geometry of the particles. Thus, particles with uneven surfaces will create very different pores compared to pores created between two well defined straight particles.

In horticultural greenhouse systems water is often applied in a short period of time such as in ebb-flood systems or locally as when using drip irrigation. Thus, the media must be able to absorb water fast and be able to distribute the water in the medium. The physical quality of a substrate is thus related to its ability to adequately store and supply air and water to plants grown in closed containers. The storage and supply of air and water are controlled by pore size abundance, tortuosity and continuity (Allaire et al., 1996).

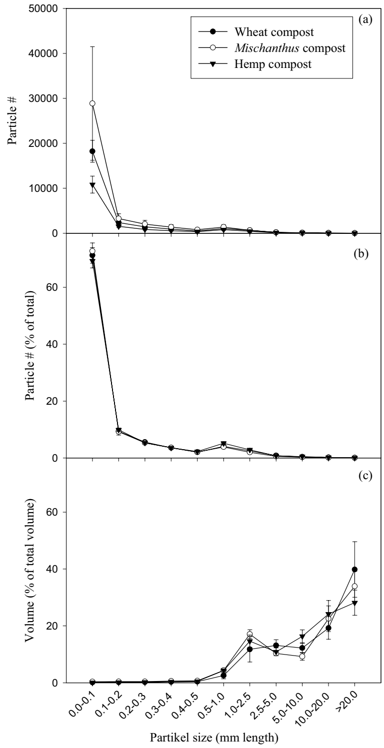

Fig.3. Particle size distribution of Wheat compost: wheat straw and clover-grass hay (●), *Mischanthus* compost: wheat straw, *Mischanthus* straw and clover-grass hay (○), and hemp compost: wheat straw, hemp straw and clover-grass hay (▼). Results are means of five replicates and bars show the standard deviation. (a) Total number of particles, (b) number as percentage of total number and (c) volume of particles.

Hence, particle size alone is not suitable when describing the media. The geometry of the particles is a key factor in determining the gas diffusivity and pore tortuosity (Nkongolo and Caron, 1999). Volume, geometry and surface characteristics of particles influence the pore sizes and continuity and pore continuity is essential for gas diffusivity and water transport.

Determining particle size by image analysis of scanned samples provided an extra information as the single particles can be studied visually. Despite the lack of quantification, these studies revealed qualitatively the differences between the three compost types examined. The wheat and *Mischanthus* composts were mainly composed of either very small particles or defined, almost intact, straw pieces, whereas the hemp compost contained more uneven particles with a downy surface (Fig.4). No differences were found between the particle size distributions of the three composts, indicating that this might not be a suitable measure of physical properties. In addition, the software might not be sufficient sensitive to determine differences of such uneven particles as observed in the compost. The actual decomposition of cell walls in wheat, *Mischanthus* and hemp straw has been examined by scanning electron microscopy (Dresbøll and Magid, submitted) revealing the differences in anatomy between the materials and thus, the differences in post-composting appearance. *Mischanthus* and wheat straw was found to degrade into more rigid pieces whereas degradation of hemp straw revealed a more flexible structure. The effect of these differences was revealed by differences in water retention capacity in these composting experiments where the materials were mixed into compost based on wheat straw and clover-grass hay.

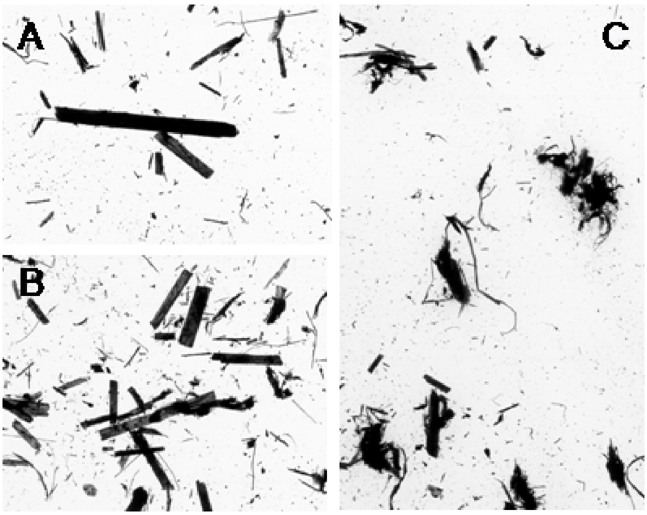

Fig.4. Scanned images of particles from a) the wheat compost, b) the *Mischanthus* compost and c) the hemp compost. When two or more particles touch, these will be measured as one particle, which can result in uncertain determinations.

3.5. Water retention capacity

The three compost types varied in water retention capacity (Fig. 5a), although the deviation between box replicates of the three compost types was large. The wheat compost contained 58% water at the zero tension level whereas the *Mischanthus* and hemp composts contained approximately 65%. When tension was initiated there was a steep initial decline in water content but below the 2 kPa tension the further decline was very small. At the tension level of 10 kPa the water content of the wheat compost was 29%, the *Mischanthus* compost had a water content of 37% and the hemp compost contained 41% water. These results differ significantly from results of water retention in peat substrates, which in general have higher water contents at the zero tension level and maintain higher water contents for a longer period with increasing suction. As expected from the scanned images the hemp compost with the irregular particles increased the capillarity of the medium and thus had a higher water retention capacity. The air filled porosity showed the inverse of the water retention increasing with increasing water tension. The highest values were found in the wheat compost and the lowest in the hemp compost (Fig. 4b). However, according to Nkongolo and Caron (1999) water retention and air filled porosity is not sufficient to describe the properties of growing media, gas exchange characteristics such as pore tortuosity and gas relative diffusivity should also be included. An ideal growing medium must have a total pore space of more than 85% (Gruda and Schnitzler, 2004). All composts in these studies had a total pore space above 90% fulfilling these requirements. In these studies gas exchange characteristics were not measured, still, the visualisation of the particles did increase the understanding of physical properties of growing media.

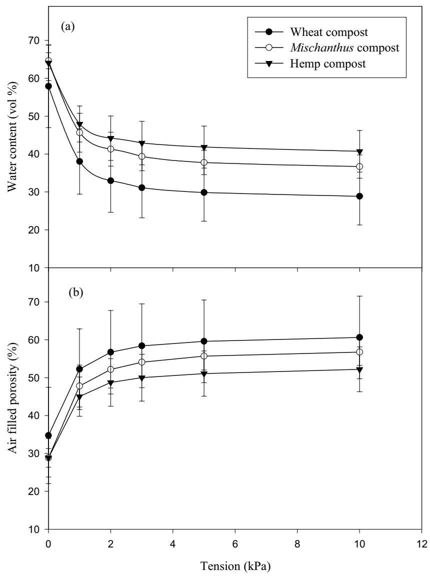

Fig.5. Water retention capacity and air filled porosity of the three different compost types. Wheat compost: wheat straw and clover-grass hay (●), *Mischanthus* compost: wheat straw, *Mischanthus* straw and clover-grass hay (○), and hemp compost: wheat straw, hemp straw and clover-grass hay (▼). Results are means of five replicates and bars show the standard deviation.

Conclusion

Knowledge of the physical properties of plant material is essential when creating a plant-based growing medium. The particle size distribution was shown not to be a good indicator of the physical properties of composted plant residues, as the substantial differences in water holding capacity was not reflected in the particle size distribution. The scanned compost samples revealed large differences in particle geometry. Hemp-based compost was shown to have more uneven particle surfaces due to the flexible structure of the decomposed hemp straw and the external bast fibres. Thus, the geometry of particles and the pores created are equally important in determining suitability of plant-based growing media.

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