Microbial activity and biomass of peats in relation to the intrinsic organic matter composition, pH, moisture, and C and N inputs

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Dedicated to my parents and my sister, the late Elsabet Amha

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Abstract

Excessive decomposition of organic matter (OM) from the potting media (e.g. peat) is known to influence plant growth by decreasing the total porosity, altering the chemical properties (pH, electrical conductivity), and releasing organic compounds that might have phytotoxic or stimulating effects. When peats are used as constitutes of the potting media, they should, therefore, maintain stability during plant production. In this study, twenty peat samples from Estonia, Finland, Germany, Ireland, Latvia, Lithuania and Sweden were evaluated for their microbial activity (measured as CO_2 and N_2O emissions) and biomass with a special emphasis to the *intrinsic organic matter composition*, *pH*, *moisture*, and *C* and *N inputs* as such information on a wide range of peat samples is largely missing from published literature. This major objective was, therefore, addressed by conducting the following six independent experiments.

- In the method comparisons study (chapter 2), five physiological and biochemical methods were compared to find out method(s) that acceptably measure(s) microbial activity and biomass in a wide range of peat samples. Based on the computed *r*-values and proportionality factor (k_{EC} of 0.436 ± 0.09), the fumigation extraction and substrate-induced respiration methods were found to be reliable methods to estimate microbial biomass-C. However, arginine ammonification, fumigation incubation, and N-stability methods showed poor correlations with basal respiration and other physicochemical properties of peats.
- In the intrinsic organic matter fractionation study (chapter 3), organic carbon (OC) extracted in hot-water bath (80 °C for 16 h) using water or salt as extractant showed strong correlations with long-term evolved CO_2 and biomass-C indicating both can be used as indicator to estimate microbial activity and biomass in peat samples. It should be noted, however, that the salt extracted OC showed less deviation within replications than water extracted OC suggesting a higher reproducibility of the former data. Evolved CO_2 over six months correlated poorly with the von Post humification degree, %N, pH, organic matter content, and the C/N ratio indicating activity in *Sphagnum* dominated peats cannot be predicted from these variables.
- In the selective inhibition study (chapter 4), contributions of fungi and bacteria to evolved CO_2 were tested. It was found that the inhibitor additivity ratio (IAR) and the fungal-to-bacterial ratio in the tested peats ranged widely from 0.76 to 1.48 and from 0.46 to 9.96, respectively. The higher IAR value in some peat samples (mainly in the mesotrophic peats) indicating non-target inhibition by the added antibiotics (streptomycin and cycloheximide). Fungal contributions were higher in the *eutrophic* and *oligotrophic* peats compared to the *mesotrophic* and *transitional* peat types, suggesting microbial community structures in peats, at least, partly, are influenced by the peat-forming environments.
- In the additives study (chapter 5), increasing the pH of all peat samples from 4.5 to 5.5 units markedly increases evolved CO_2. However, microorganisms in the weakly humified peats responded strongly for the pH increases from 5.5 to 6.5 (compared to the moderately and

strongly humified peats) to indicate that these peats cannot maintain their stability at a higher pH. The responses of microorganisms to cellulose additions were positive (15–146% of control) although the increases over 28 d were considerably lower than glucose (78–514%) and arginine (36–294%) treated samples to suggest that the inclusions of cellulosic rich potting constitutes (e.g. wood bark, fibers, and saw dust) into peat samples may have little effect on the bio-stability of the final mixes. However, cumulative CO_2 emission in the N treated peat sample was generally lower than in the control (decreased by 7.3 to 75.8%) regardless of the types of N-forms used (KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3).

- In the denitrification study (chapter 6), basal and potential denitrification rates were substantially increased by 3.6–14 and 1.4–2.3-fold, respectively, when the initial pH (4.3–4.8) was raised to 5.9–6.5 units. Emissions of (N_2O+N_2)-N from most peats were markedly increased by the addition of 0.15 g NO_3^-–N L^{-1} dry peat but further additions had no effect. Denitrification rates were increased by increasing glucose concentration suggesting that the activity of denitrifiers in all peat types was limited by the low availability of easily decomposable C source. Increasing moisture contents of all peats from 40 to 50% water filled pore space (WFPS) did not significantly ($p > 0.05$) increase (N_2O+N_2)-N emissions. However, a positive effect was observed when the moisture contents were increased from 60 to 70% WFPS in the eutrophic peat, from 70 to 80% in the transitional, from 80 to 90% in the oligotrophic and from 70 to 90% in the mesotrophic peats.
- In the physical and chemical properties study (chapter 7), the tested peats had substantial amounts of water volume (W_V; 43.3 to 82.1 % *v/v*) but their respective mean water buffering capacities were low (<8 % of W_V) to suggest that these peats cannot deliver enough water to the cultivated plant once the easily available water (i.e., water held between 1 and 5 kPa; 17.2 to 42.3 % *v/v*) is gone. The greater percentages of water in the tested peats were held at higher moisture tension (>10 kPa), which is mostly become less available for root uptake. Mineral contents (NH_4^+–N, NO_3^-–N, P, Mg, K, Ca, Mn and Fe) in most peats were considerably low and could be neglected during fertilization. However, the higher resource availability indices (C/N, C/P, N/P and N/K) in these peats may play greater roles in determining microbial mediated N and C transformations during plant cultivation.
- Overall, the whole peat samples were broadly classified into three distinct groups using the hierarchical cluster analysis: the Irish and two of German peats produced the lowest CO_2 while most peats from Finland produced the highest CO_2. With few exceptions, peats from the Baltic States occupied the middle ranges. Excessive decomposition of organic matter in the Finish peats might have unintended consequences if these peats are used for long-term pot plant production. With regard to botanical composition, peats containing *Sphagnum imbricatum* produced the lowest CO_2 and *S. angustifolium* dominated peats mostly produced the highest CO_2.

Key words: carbon dioxide, denitrification, fungal-to-bacterial ratio, organic matter, peat

Kurzfassung

Eine starke Zersetzung der organischen Substanz von Kultursubstraten (z. B Torf) führt zu einer Abnahme des Gesamtporenvolumens, verändert chemische Eigenschaften (pH-Wert, elektrische Leitfähigkeit) und setzt neue organische Verbindungen mit phytotoxischer oder stimulierender Wirkung frei. Diese Prozesse wirken sich auf das Pflanzenwachstum aus. Torfe als Bestandteile von Kultursubstraten sollten daher ihre Stabilität während der Pflanzenproduktion beibehalten. In dieser Studie wurden zwanzig Torfproben aus Estland, Finnland, Deutschland, Irland, Lettland, Litauen und Schweden bezüglich ihrer mikrobiellen Aktivität und Biomasse bewertet (gemessen als CO_2 und N_2O-Emissionen). Die Untersuchungen hatten folgende Schwerpunkte: inhärente Zusammensetzung der organischen Substanz, pH-Wert, Wassergehalt, C- und N-Gaben. Über diese Zusammenhänge gibt es für ein breites Spektrum an unterschiedlichen Torfen in der Fachliteratur keine Informationen. Das Ziel der Untersuchungen sollte mit den folgenden sechs unabhängigen Experimenten erreicht werden.

- In dem Experiment „Methodenvergleich“ (Kapitel 2) wurden fünf physiologische und biochemische Methoden verglichen. Ziel war es, die Methode(n) zu finden, die in akzeptabler Weise die mikrobielle Aktivität und Biomasse in einem breiten Spektrum von Torfen angibt. Basierend auf den berechneten r-Werten und dem Proportionalitätsfaktor (K_{EC} von 0,436 ± 0,09), konnten die Methoden „Chloroform-Fumigation-Extraktion“ und „Substrat-induzierte Atmung“ als zuverlässige Verfahren eingestuft werden, um die mikrobielle Biomasse (C_{mik}) abzuschätzen. Demgegenüber zeigten die Methoden „Arginin-Ammonifikation, „Chloroform-Fumigation-Inkubation“ und „N-Stabilität“ nur eine geringe Korrelationen mit der Basalatmung und anderen physikalisch-chemischen Eigenschaften der Torfe.

- In dem Experiment „Fraktionierung der organischen Substanz“ (Kapitel 3) wurde die organische Substanz im heißen Wasserbad (80 °C für 16 h) mit Wasser oder Salzlösung extrahiert. Die Ergebnisse zeigten eine starke Korrelation mit dem langfristig freigesetzten CO_2 und der mikrobiellen Biomasse (C_{mik}); sie können daher beide als Indikator verwendet werden, um die mikrobielle Aktivität und Biomasse in Torfen zu schätzen. Allerdings traten bei der Extraktion der organischen Substanz mit einer Salzlösung geringere Abweichungen zwischen den Wiederholungen auf als bei Extraktion mit Wasser. Dieses weist auf eine bessere Reproduzierbarkeit der Daten bei Verwendung einer Salzlösung hin. Das in sechs Monaten freigesetzte CO_2 war schlecht mit dem von Post-Humifizierungsgrad, % N, pH-Wert, Gehalt an organischer Substanz, und dem C/N-Verhältnis korreliert. Die mikrobielle

Aktivität in *Sphagnum*-dominierten Torfen kann daher nicht mit diesen Variablen vorhergesagt werden.

- In dem Experiment „Selektive Hemmung“ (Kapitel 4) wurden die Beiträge von Pilzen und Bakterien an den CO_2-Emissionen untersucht. Es wurde festgestellt, dass das Additivitätsverhältnis der Hemmstoffe (inhibitor additivity ratio, IAR) und das Verhältnis Pilze-zu-Bakterien in den getesteten Torfen in einem weiten Bereich von 0,76 bis 1,48 bzw. 0,46 bis 9,96 lagen. Der hohe IAR-Wert in einigen Torfen (hauptsächlich in den mesotrophen Torfen) zeigt an, dass die zugesetzten Antibiotika nicht ausschließlich zu einer Hemmung von Bakterien und Pilzen geführt haben. Der Beitrag von Pilzen war in eutrophen und oligotrophen Torfen im Vergleich zu den mesotrophen Torfen und Torfen aus Übergangsmooren höher, was darauf hindeutet, dass die Zusammensetzung mikrobieller Gemeinschaften in Torfen zumindest teilweise durch die torfbildende Umgebung beeinflusst wird.

- In dem Experiment Zusatzstoffe (Kapitel 5) führten Kalkung und pH-Wert-Zunahme von 4,5 auf 5,5 bei allen Torfproben zu einer deutlich Zunahme der CO_2-Freisetzung. Allerdings reagierten die Mikroorganismen in schwach humifizierten Torfen im Vergleich zu den mäßig und stark humifizierten Torfen besonders stark auf den pH-Anstieg von 5,5 bis 6,5. In den schwach humifizierten Torfen führt die Zunahme des pH-Wertes zu einer Abnahme der Stabilität der organischen Substanz. Der Zusatz von Cellulose erhöhte die Aktivität der Mikroorganismen (15 bis 146% im Vergleich mit der Kontrolle), allerdings war die Zunahme innerhalb von 28 Tagen erheblich niedriger als nach dem Zusatz von Glucose (78–514%) und Arginin (36–294%). Der Zusatz cellulosereicher Komponenten in torfbasierten Kultursubstraten (z. B. Holz, Rinde, Fasern und Sägemehl) wird daher kaum Auswirkungen auf die Stabilität der fertigen Mischungen haben. Demgegenüber waren nach Zusatz von N, unabhängig von der Art der verwendeten N-Form (KNO_3, $(NH_4)_2SO_4$ und NH_4NO_3), die kumulierten CO_2-Emissionen in der Regel niedriger als in der Kontrollgruppe (sie sanken um 7,3 bis 75.8%).

- In dem Experiment „Denitrifikation“ (Kapitel 6) wurden basale und potenzielle Denitrifikationsraten um die Faktoren 3,6-14 und 1,4-2,3 erhöht, wenn der anfängliche pH-Wert von 4,3-4,8 auf 5,9 bis 6,5 Einheiten angehoben wurde. Bei den meisten Torfen wurden die Emissionen von (N_2O + N_2)-N durch den Zusatz von 0,15 g NO_3-N L^{-1} trockener Torf deutlich erhöht, aber höhere Zusätze hatten keine weitere Wirkung. Die Denitrifikationsraten nahmen mit steigender Glucosekonzentration zu, was darauf

hindeutet, dass die Aktivität der Denitrifikanten in allen Torfarten durch die geringe Verfügbarkeit von leicht abbaubaren C-Quellen begrenzt war. Bei allen Torfen führten steigende Wassergehalte von 40 auf 50% des mit Wasser gefüllten Porenraum (WFPS) nicht zu einer signifikanten ($p > 0.05$) Zunahme (N_2O+N_2)-N-Emissionen. Allerdings wurde eine Zunahme beobachtet, wenn der Wassergehalt in eutrophen Torfen von 60 auf 70% WFPS, in Torfen aus Übergangsmooren von 70 auf 80%, in oligotrophen Torfen von 80 auf 90% und in mesotrophen Torfen von 70 auf 90% WFPS erhöht wurde.

- In dem Experiment „Physikalische und chemische Eigenschaften“ (Kapitel 7) zeigten die untersuchten Torfe eine hohe Wasserkapzität (WV; 43,3 bis 82,1% v/v). Die mittleren Pufferkapazitäten für Wasser waren allerdings gering (<8% der WV), so dass diese Torfe nach Verbrauch des leicht verfügbaren Wassers (d. h. Wasser zwischen 1 und 5 kPa; 17,2 bis 42,3% v / v) nicht genug Wasser nachliefern können, um die Kulturpflanzen zu versorgen. Die größere Menge Wasser in den untersuchten Torfen lag bei höheren Wasserspannungen (> 10 kPa) vor und ist für die Aufnahme durch die Wurzel weniger leicht verfügbar. Die Mineralstoffgehalte (NH_4^+-N, NO_3^--N, P, Mg, K, Ca, Mn und Fe) waren in den meisten Torfen niedrig und können bei der Düngung vernachlässigt werden. Allerdings könnten die hohen Nährstoffverhältnisse (C/N, C/P, N/P und N/K) in diesen Torfen wegen der mikrobiell vermittelten N- und C-Transformationen während der Kultivierung der Pflanzen eine größere Rolle spielen.

- Insgesamt wurden alle Torfe mit Hilfe der hierarchischen Clusteranalyse grob in drei Gruppen klassifiziert: Torfe aus Irland und zwei der vier Torfe aus Deutschland setzten die geringsten CO_2-Mengen frei, während die meisten Torfe aus Finnland die höchste CO_2-Freisetzung hatten. Mit wenigen Ausnahmen lagen die Torfe aus den baltischen Staaten im mittleren Bereich. Starker Abbau der organischen Substanz in finnischen Torfen könnte unbeabsichtigte Folgen haben, wenn diese Torfe für eine Topfpflanzenproduktion mit langen Kulturzeiten verwendet werden. Die geringste CO_2-Freisetzung fand in Torfen statt, die *Sphagnum imbricatum* enthalten, während Torfe, die von *S. angustifolium* dominiert waren, meist die höchste CO_2–Freisetzung hatten.

Key words: Kohlendioxid, Denitrifikation, Pilz-to-Bakterien-Verhältnis, organische Substanz, Torf

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Abbreviations

AA	Argenine ammonification
ATP	Adenosine triphosphate
D_{BD}	Dry bulk density
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EC	Electrical conductivity
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EN	European norm
F:B	Fungal-to-bacterial ratio
FE	Fumigation extraction
FI	Fumigation incubation
GC	Gas chromatograph
GWC	Gravimetric water content
IAR	Inhibitor additivity ratio
IPS	International peat society
k_{EC}	Extractable part of microbial biomass carbon after fumigation
MB-C	Microbial biomass carbon
MIRR	Maximum initial respiratory response
N_{min}	Mineral nitrogen
OC	Organic carbon
odw	Oven dry weight
OM	Organic matter
P_s	Total pore space
pH	A negative decimal logarithm of the hydrogen ion activity in a solution
PLFA	Phospholipid fatty acid
PMN	Potentially mineralizable nitrogen
qCO_2	Respiratory quotient
SI	Selective inhibition
SIR	Substrate-induced respiration
TCD	Thermal conductivity detector
TOC	Total organic carbon
WFPS	Water filled pore space

Units

%	Percent
°C	Degree Celcius
µ	Micro (10^{-6})
cm	Centimeter
cm^3	Cubic centimeter (= mL)
d	Day(s)
g	Gram
h	Hour
k	Kilo (10^3)
kPa	Kilo Pascal
L	Liter
m	Meter
M	Molar
mg	Milligram (10^{-3} g)
min	Minute
mL	Milliliter
mm	Millimeter
ng	Nano (10^{-9}) gram
ppm	Parts per million
rpm	Revolution per minute
%, m/m	Percent expressed in mass-to-mass ratio
%, v/v	Percent expressed in volume-to-volume ratio
%, w/v	Percent expressed in weight-to-volume ratio

Symbols (Elements/Compounds)

C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
$Ca(NO_3)_2$	Calcium nitrate
C_2H_2	Acetylene
$CHCl_3$	Chloroform
CO_2	Carbon dioxide
CO_3	Carbonate
$CuCl_2$ - $2H_2O$	Copper(II) chloride dihydride
Fe	Iron
$FeCl_3$-$4H_2O$	Iron chloride (III) tetrahydride
HCl	Hydrochloric acid
H_3BO_3	Boric acid
H_3PO_4	Phosphoric acid
He	Helium
K	Potassium
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Dipotassium phosphate
KNO_3	Potassium nitrate
K_2SO_4	Potassium sulphate
Mg	Magnesium
$MgSO_4$-$7H_2O$	Magnesium sulfate heptahydrate
Mn	Manganese
$MnCl_2$ - $4H_2O$	Manganese chloride tetrahydrate
N	Nitrogen
$(NH_2)_2CO$	urea
NH_3	Ammonia
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
NH_4^+–N	Ammonium-nitrogen

NH_4NO_3	Ammonium nitrate
Ni	Nickel
NO	Nitric oxide
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_3^-–N	Nitrate-nitrogen
P	Phosphorous
$ZnCl_2$	Zinc Chloride

1. General introduction

1.1. Peat

Shotyk (1988) defined peat as a light brown to black organic material formed under waterlogged conditions from the partial decomposition of mosses and other bryophytes, sedges, grasses, shrubs, or trees. The rate of peat accumulation may vary considerably between the peat-forming ecosystems (i.e., peatlands) with an overall accumulation rate of 0.5 mm $year^{-1}$ (Gorham, 1991). Some of these peat deposits are being extracted and used principally in horticulture, agriculture, domestic heating, and energy generation. The International Peat Society (IPS) survey report indicated that the annual total production of peat worldwide is amounted to ≈90 million cubic meters (EPAGMA, 2005), of which 40% is designated for horticultural uses. Although peats are regarded as stable/uniform organic materials, their respective physical, chemical, and microbiological properties could vary considerably depending on the *degree of decomposition*, *botanical composition*, and the *peat-forming environments* (Bohlin et al., 1989; Clymo, 1983; Moore et al., 2007; Shotyk, 1988; Steinmann and Shotyk, 1997).

1.1.1. The degree of decomposition in peat

Although the degree of decomposition is not a clearly defined term (Preston et al., 1987), it generally measures the relative quantity of one of the chemical or physical characteristics of organic soils (e.g. peats) that changes with advanced decomposition (Boelte, 1969). It can be measured using various techniques including the von Post scale (von Post, 1924), the visual fiber estimate (Farnham and Finney, 1965), the unrubbed and rubbed fiber (Stanek and Silc, 1977), pyrophosphate index (Kaila, 1956), reflectivity percentage (Grandmaison and Laflamme, 1986), and others. However, the von Post scale (von Post, 1924) is one of most widely used methods for measuring peat decomposition. In this qualitative method, a small amount of moist peat sample is squeezed by hand and the value determined from the color of the running water and from the nature of the residue. On the von Post scale, peats can be classified into 10 classes: H1 (completely unhumified) to H10 (completely humified) where weakly to

moderately humified peats (H2–H5) are the most preferred ones in horticulture (Daigle and Gautreau-Daigle, 2001; Holden and Ward, 1997). The term humification degree is often used in horticulture to describe the von Post humification scale.

1.1.2. The peat-forming environment

The characteristics of peats are greatly influenced by the peat-forming environments (Charman et al., 1994; Grumpelt, 1991; Shotyk, 1988). On the trophic levels, peats can be classified as *oligotrophic*, *mesotrophic*, *eutrophic* and *transitional* types. The oligotrophic peat generally evolved from ombrotrophic *bog* and is entirely depended on precipitation for its nutrient source; whereas, the mesotrophic and eutrophic peats developed from *fens* that receive minerals mostly from ground and surface water (i.e., minerotrophic types). The transitional peat formed in the intermediate mire (e.g. fen-to-bog interval) where neither the precipitation nor surface/ground water dominates the nutrient balance. The resistance of *Sphagnum* remnants to decomposition and their long-term accumulation as peat is favoured by their habitat conditions as well as by the peculiar chemistry of *Sphagnum* plants. In general, an oxopolysaccharide (Painter, 1983) and specific secondary compounds such as uronic acids and polyphenols that suppress soil heterotrophs and extracellular enzyme activity characterize *Sphagnum* litter (Freeman et al., 2004).

1.1.3. The botanical composition of peat

Plant remains in peats are in some ways analogous to the mineral constitutes of rocks (Shotyk, 1988), and provide valuable information about the environment of accumulation. Peats are often composed of the remnants of different plant genus. However, more than half of the world's peat originated from *Sphagnum* (Clymo and Hayward, 1982), and representing 10-15% of the terrestrial carbon (C) stock, with more C held in dead and living *Sphagnum* than is fixed annually by all terrestrial vegetation. Watson (1981) described the common *Sphagnum* species in the bogs as: (i) *S. cuspidatum, S. recurvum,* and *S. subsecundum* in the pools (wettest site), (ii) *S. fuscum, S. rubellum,* and *S. russowii* on the hummocks (drier site), and (iii) *S. magellanicum, S. palustre, S. papillosum, S. subnitens,* and *S. tenellium* in hollows-intermediate zones

between pools and hummocks. Bog surface waters are generally acidic (pH ≈ 4; Clymo, 1983) and plant nutrients are in relatively short supply. Fens, on the other hand, are mostly dominated by *Carex* species. *Carex* peat contains much less cellulose and hemicellulose than *Sphagnum* (Bohlin et al., 1989), which makes it more degradable than *Sphagnum* peat. Such differences in the botanical origin of the plant litter could greatly influence the chemical composition, biodegradability, and potential of peats for producing CO_2 and CH_4 (Moore et al., 2007).

1.2. Peat in horticulture

Peat moss is one of the most widely used organic potting media for crop production, either by itself or in combination with other materials. The significance of peat in horticulture lies mostly in its unique combination of physical properties that enable it to retain large volumes of water, air and plant nutrients in readily available forms (Robertson, 1993). In addition to these unique physical properties, the chemical property of peat (e.g. low pH and low in nutrient contents) gives an excellent precondition for its wide application as a potting medium for a variety of greenhouse and nursery plants (Reinikainen, 1997). However, all peat types may not always meet such quality. In the commercially available potting media, therefore, peats of different origins are often mixed to create stable potting media for plant growth (Prasad and Maher, 2008). Different additives (e.g. lime, fertilizer, binding agents, buffering materials, wetting agents, biocontrol agents, dye, etc) can also be added to improve the quality of peat-based media (Schmilewski, 2003).

The basic functions of potting media (e.g. peats) are primarily to provide mechanical support (Brückner, 1997), and to deliver water, oxygen and nutrients to the greenhouse and nursery crops (Bohlin and Holmberg, 2004). Microbial degradation of potting media is, therefore, unwelcome as it causes a change in (i) physical structure (e.g. water and air capacity), (ii) chemical behavior (e.g. pH, electrical conductivity, change in cations and anions exchange capacity), and (iii) nutrient balances (because of mineralization and/or immobilization of nutrients) (Lamaire, 1995; Prasad and O'Shea, 1997; Verhagen, 2009). The activity of some thermophilic microbes (especially during self-heating) might also produce phytotoxic (Wever, 1991) and water repellent colloids

(Puustjärvi, 1983) that negatively influencing the water retention of peat-based media. Furthermore, excessive degradation of OM in the container might incur costs to the nurseryman, as such containers need refilling before marketing (Aendekerk, 1997). Results from the aforementioned studies are, therefore, suggesting that decomposition of OM in the potting media is less desirable during plant growth. However, a number of factors may influence microbial degradation of peats during plant growth including:

1.2.1. Intrinsic organic matter composition

The rates of microbial degradation of peats are dependent on the fractions of the intrinsic OM that heterotrophic microorganisms can easily use as carbon and energy sources (Davidson et al., 1987). Although the content of OM in peat could reach up to 100% of the respective dry mass (Clymo, 1983), its chemical composition may vary greatly depending on the botanical composition, degree of decomposition, and the peat-forming environment (Bohlin et al., 1989; Shotyk, 1988; Steinmann and Shotyk, 1997). The work of Cocozza et al. (2003) indicates that the typical abundances of carbon, hydrogen, and oxygen in peats are in the range 50–60, 5–6 and 30–40%, respectively, where the latter element generally decreases with increasing humification degree. Organic matter in a strongly humified peat is mostly a recalcitrant type (Bohlin et al., 1989) and degrades less in the container as compared to a weakly humified one. However, OM in peat or soil could display a large degree of heterogeneity ranging from active to passive pools (Piccolo, 2001) to suggest that not all OM in peat/soil equally available for microbial utilization. It is, therefore, assumed that fractionation of the intrinsic OM into different components might produce a simple and persuasive indicator about the activity and biomass of microbes in peats.

1.2.2. Microbial characteristics

The biodegradability of the intrinsic OM in peat could be largely associated with its microbial characteristics (population size, activity, and composition). Various authors including Anderson and Domsch (1975), Bailey et al. (2002) and West (1986) indicating that microorganisms are responsible for the majority of OM decomposition and mineralization in soils, and preserve energy and nutrients in their biomass. The

relationship between CO_2 evolution and the size of microbial biomass is significantly affected by the fungal-to-bacterial biomass ratio (Sakamoto and Oba, 1994). Apart from bacteria and fungi, soil microorganisms also encompass various genera and species of archaea and protozoa. However, the characteristics of microorganisms in peats differ widely between the degree of decomposition and the peat-forming environments. Studies have shown that the fungi associated with *Sphagnum* are capable of decomposing a large variety of substrates such as lignin and cellulose (Thornmann et al., 2002), and *Penicillium* spp., *Mortierella* spp., and *Trichoderma* spp. are among the common colony forming units in weakly humified peats (Tahvonen and Kemppainen, 2008). Dedysh et al. (2006) characterized the bacterial community in an acidic Sphagnum peat bog as *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Alphaproteobacteria*, *Verrucomicrobia*, *Deltaproteobacteria*, and *Planctomycetes*. Fungi typically have larger biomass and C:N ratios than bacteria and therefore may be more capable of decomposing nutrient poor litters and soil organic matter (Paul and Clark, 1996). Soil fungi typically have larger assimilation: respiration ratios than bacteria (Sakamoto and Oba, 1994). In terms of biomass, heterotrophic bacteria, fungi, amoeba, and microalgae are the dominant groups of microorganisms in European peatlands (Mitchell et al., 1999). The relative importance of heterotrophic organisms (73-95%) and the strong dominance of testate amoeba in the protozoan biomass were characteristic for *Sphagnum* dominated peatlands. The native microbial community could, however, be altered during storage and cultivation seasons. Moreover, introduction of mycorrhiza and biocontrol agents to improve plant growth can alter the initial microbial characteristics.

1.2.3. pH and nutrition

The value of pH in *Sphagnum* originated peat is low (acidic), which is partly attributed to the presence of a relatively higher concentration of dissolved organic acids (Steinmann and Shotyk, 1997). The inclusions of lime, fertilizer and other additive materials into peats could, however, alter the initial pH, nutrient availability and the activity of microorganisms. Strongly humified peat may have a greater buffering capacity and therefore be more stable with regard to pH and physical structure than a younger peat (Maher and Prasad, 2004). Liming generally increases pH and usually

improves the bacteria growth over fungi. Although N-fertilization does generally increase the availability of N in the soils, its effects on microbial respiration can be positive (Recous et al., 1990), negative (Aerts and Toet, 1997; Bridgham and Richardson, 1992) or neutral (Wickramasinghe et al., 1985). However, the responses of microorganisms to pH and nutrition are largely unknown for horticultural peats.

1.2.4. Moisture and air contents

Peat-based medium holds a higher amount of water at lower water tensions compared to other growing media, which is partly explained by the possession of original cellular structure comprising a series of hollow cells (ca. 10 μm) (Robertson, 1993). The initial water-air dynamics of peats could, however, be changed during crop cultivation as results of settling and decomposition of the intrinsic OM. Microbial respiration in some organic soils or growing media may reach as much as ten times higher than the upper value for mineral soils (Glinski and Stepniewski, 1985), which in turn decreases the availability of oxygen to the plant root. Naasz et al. (2008a) have studied the availability of oxygen in *Sphagnum* peat medium using a one-dimensional transfer coupled model and observed a 23% decline in oxygen availability in the rhizosphere when the rate of microbial respiration and the volumetric water content of peat adjusted to 30 mg O_2 m^{-3} s^{-1} and 60% v/v, respectively. However, increasing microbial respiration rate from 30 to 120 mg O_2 m^{-3} s^{-1} considerably decreased the availability of oxygen in the rhizosphere by 60%. Overall, a higher hydraulic conductivity in peat observed at a volumetric water content >45% while a higher oxygen diffusivity at a volumetric water content <70% (Naasz et al., 2008b). Volumetric water contents between 45 and 70% considered to be a "non-limiting" range where a little change above or below this range could result in a rapid fluctuation of water and air in the vicinity of plant root (rhizosphere).

1.2.5. Plant root

Plants yield different amount of root exudates into the rhizosphere as organic inputs (Merbach et al., 1999), which could alter the composition of organic compounds and microorganisms in peat-based media. According to Kuzyakov (2002), about 15 to 25%

of belowground allocated C will be exuded out by the active root parts and cause fast microbial turnover in the rhizosphere. Although the composition of root exudates varied between species (Merbach et al., 1999), the main fractionated water-soluble root borne C compounds are carbohydrates like glucose and fructose; organic acids and amino acids (Hütsch et al., 2002). Moreover, dead root cap cells and cell lysates contribute for increased C source in the rhizosphere (Marschner, 1995). These C-compounds would affect the number and composition of microorganisms in the rhizosphere, which in turn influence the stabilization or decomposition of OM (Marschner and Kalbitz, 2003). According to Bodelier et al. (1997), the number of microorganisms in the rhizosphere is 19 to 32 times larger than in the root-free soil.
Overall, the effect of rhizodepositions (organic substances exuded out to rhizosphere from plant roots; Kuzyakov, 2002) is assumed to be high in the planted potting medium, as the whole root ball would soon be covered by plant root.

1.3. Why do we measure microbial activity and biomass

As part of quality assurance (VDLUFA, 2006), at least, in Germany, the stability of potting media for N-mineralization and immobilization is checked through the N-stability test. Potting media that qualify this test, however, often behave differently during plant cultivation (Amha, 2006). This is because; the N-stability test does not consider, among others, the availability of easily decomposable organic C for microbes through rhizodepositions. This method has also a number of flaws (e.g. the use of high moisture content, high N addition, and small sample size) that in turn limit its ability to predict the stability of N in the potting media during plant growth. Therefore, direct measurements on microbial activity and biomass under different conditions are indispensible to understand the transformation of organic and inorganic substances (Kandelar, 2007) in the potting media as they *per se* represent a sink and a source for plant nutrients. Moreover, the activity and biomass data could help us to understand the response of microbiota to management changes (fertilization, irrigation, chemical application, environmental conditions, etc). Additional merits of characterizing the biological properties of potting media were reviewed by Alsanius and Wohanka (2009). Although the applicability of the existing methods for peats are largely untested, microbial activity and biomass in forest, grassland and agricultural soils can

be measured by a direct microscopy, substrate-induced respiration (SIR), selective-inhibition (SI), fumigation incubation (FI), fumigation extraction (FE), arginine ammonification (AA), ATP extraction, ergosterol extraction, phospholipid fatty acid (PLFA), and others.

1.4. Objectives

Twenty peat samples were collected from different areas of Finland, Germany, Ireland, Sweden, and the Baltic States (Estonia, Lithuania and Latvia) with ***a general objective of investigating the microbial activity and biomass of peats in relation to the intrinsic OM composition, pH, moisture, and C and N inputs*** as this information over a wide ranges of peat samples is largely missing from published literature. The specific objectives are:

- to compare the suitability of five physiological and biochemical methods (AA, SIR, FI, FE and N-stability) in estimating the microbial activity and biomass of peat samples (chapter 2);
- to fractionate, quantify and examine the relationships between OM fractionates and microbial activity and biomass measured over long-term incubation experiment (chapter 3);
- to evaluate the responses of peat microorganisms to additives used in horticulture (N-fertilizers, liming and binding materials) (chapter 4);
- to partition fungal and bacterial contributions to microbial respiration using a selective inhibition technique, and to optimize the amount of antibiotics (streptomycin and cycloheximide) and glucose to be added to measure the fungal-to-bacterial ratio in peat samples (chapter 5);
- to measure denitrification rates from peats differing in pH, and moisture, nitrogen and carbon contents using the acetylene inhibition technique (chapter 6); and
- to evaluate the physical and chemical properties, and botanical composition of peats used in horticulture (chapter 7).

2. Comparison of physiological and biochemical methods for assessing microbial activity and biomass of peats

2.1. Abstract

A method that acceptably predicts the biodegradability of organic potting media (e.g. peats) is of a great importance to asses microbially mediated processes during crop cultivation. In this study, 20 peat samples from seven European countries were evaluated for their microbial activity and biomass using the substrate-induced respiration (SIR), arginine ammonification (AA), fumigation incubation (FI), fumigation extraction (FE) and N-stability methods. Basal respiration (measured as evolved CO_2), microbial biomass-C estimated by the SIR ($MB\text{-}C_{SIR}$) and the FE ($MB\text{-}C_{FE}$) methods ranged from 24 to 128 CO_2-C g^{-1} dry peat d^{-1}, from 276 to 1802 µg C g^{-1} dry peat, and from 397 to 2172 µg C g^{-1} dry peat, respectively. Basal respiration showed strong correlations with ($MB\text{-}C_{SIR}$; $r = 0.89$) and FE ($MB\text{-}C_{FE}$; $r = 0.83$) but not with FI ($MB\text{-}C_{FI}$; $r = 0.35$), AA ($r = -0.61$) and N-stability ($r = -0.16$) to suggest that FE and SIR are the only reliable methods to estimate microbial biomass in a wide range of peat samples. The equation: MB-C = 2.372 ± 0.059 (extractable C-flush; E_C) gave an overall proportionality factor (k_{EC}) of 0.422, which is nearly similar to the mean value of individual k_{EC} computed as the ratio of E_C to $MB\text{-}C_{SIR}$ (0.436 ± 0.09). Basal respiration correlated poorly with von Post humification degree (H), %N, pH, organic matter content, and the C/N ratio indicating that microbial activity in *Sphagnum* dominated peats cannot be predicted from these variables.

Keywords: arginine ammonification, basal respiration, fumigation extraction, fumigation incubation, peat-forming environment, substrate-induced respiration

2.2. Introduction

Techniques for monitoring the biodegradation of organic matter from the rate of efflux of CO_2 were pioneered by Waksman (1932), and are widely used in soil microbial studies. This is because microbial activity in most soils (measured as evolved CO_2) has a close relationship with microbial biomass estimated by different methods (Martens, 1987). For instance, in the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978), the maximum initial respiratory response following glucose addition serves as a basis to estimate the metabolically 'active' biomass in different soils. CO_2 increase due to fumigation is also used to measure chloroform sensitive 'total' microbial biomass by the fumigation incubation (FI) method (Jenkinson and Powlson, 1976; Vance et al., 1987a). Fumigation extraction (FE) method, on the other hand, measures chloroform sensitive total microbial biomass by extracting the total organic carbon (TOC) with 0.5 M K_2SO_4 solution (Vance et al., 1987b) before and after fumigation. Enzyme assay based on arginine ammonification (i.e., liberation of ammonia from the added arginine) is another method used to assess the soil microbial biomass in their actual metabolic state (Alef and Kleiner, 1987; Lin and Brookes, 1999).

Microbial activity and biomass in mineral and forest soils have been extensively studied using the aforementioned methods (Anderson and Domsch, 1978; Lin and Brookes, 1999; Martens, 1987; Vance et al., 1987a, b; West and Sparling, 1986). Nevertheless, the applicability of these methods to peats used in horticulture remained untested. Method that acceptably quantifies the biodegradability of potting media (e.g. peats) is, however, essential to evaluate microbially mediated processes during crop cultivation. The objectives of this particular work were (i) to evaluate microbial activity and biomass of horticultural peats using the SIR, FI, FE and AA methods and (ii) to examine the relationships between microbiological data and some of the selected physicochemical properties of peat samples. The N-stability test (VDLUFA, 2006) was also included in these comparisons, as it is the most widely used laboratory method in Germany to evaluate the N-balance of the potting media.

2.3. Materials and Methods

2.3.1. Peat samples

Twenty peat samples were obtained from peatlands of seven European countries (Table 1). These peatlands are being partly used as a source of peat materials for horticulture and domestic energy uses. From each site, subsamples were taken in three plastic bags and sieved separately to 5 mm. Each bag was then served as a replicate. The botanical compositions of peats were identified following Heikurainen and Huikari (1952). Briefly, air dried peat was crushed carefully with mortar and representative subsample (100-200 mL) was soaked in deionized water for a minimum of 2 h. Botanical identification was then made from the peat particles with the help of a high resolution microscope (Meiji Company, Japan). Critical identification markers such as smallest pore holes, cell structure, and mid and/or tip parts of the leaf were used to ascertain different plant remains. The degree of decomposition was determined according to the von Post humosity scale (von Post, 1924). In this qualitative method, a small amount of moist peat sample was squeezed by hand and the value determined from the color of the running water as well as from the nature of the residue.

All peats in Table 1 were also classified into *oligotrophic*, *mesotrophic*, *eutrophic* and *transition* types based on the source of water/nutrient in their respective peat-forming environments (Stewart and Kantrud, 1971). The oligotrophic peats (n = 9) evolved from ombrotrophic bogs that are entirely dependent on precipitation for their nutrient source. The mesotrophic (n = 3) and eutrophic (n = 2) peats, on the other hand, developed from fens that received mineral materials from both ground and/or surface water (Shotyk, 1988; Steinmann and Shotyk, 1997). The water balance in the transitional peat-forming environment (n = 6) is neither dominated by precipitation nor surface/ground water. Details on the physicochemical properties and botanical compositions of these peats have been reported elsewhere (Amha et al., 2010). Overall, these peats were quite variable in their dry bulk density (40–141 g L^{-1}), organic matter content (91.4–99.8%), total C (46.9–52.2%), total N (0.48–2.08%), the C/N (24–101), N/P (24–81), and C/P ratio (1194–3612).

Table 1
Details on the peat-forming environment and botanical composition of peat samples obtained from seven European countries

Peat types	Country	Area of extraction	[a]H	[b]Relevant *Sphagnum* species	[c]Peat-forming environment
1	Latvia	Turkums	3	2, 5, 8, 10	ombrotrophic bog[d]
2	Lithuania	Gabolinus	3	4, 8, 11, 15	ombrotrophic bog
3	Lithuania	-	3	1, 6, 8, 10	fen-bog transition
4	Estonia	West Estonia	3	2, 6, 8	ombrotrophic bog
5	Finland	Lapua	3	5, 8, 10, 15	ombrotrophic bog
6	Finland	Seinajoki	3	1, 10	fen-bog transition
7	Finland	Närpio	3	1, 8	ombrotrophic bog
8	Latvia	LaFlora	4	1, 6, 8, 10	ombrotrophic bog
9	Ireland	Sharagh	4	9	mesotrophic fen
10	Lithuania	15b Laukas	4	1, 3, 10, 14	fen-bog transition
11	Ireland	Brehony	4	1, 8, 9	ombrotrophic bog
12	Germany	Osnabrück	4	8, 10, 13	fen-bog transition
13	Estonia	P-27	5	8	ombrotrophic bog
14	Sweden	Drakamyr	5	8, 9	fen-bog transition
15	Germany	Vechta	5	7, 13	mesotrophic fen
16	Finland	Kikilla	5	16	eutrophic fen
17	Germany	Vehmemoor	6	9, 12	mesotrophic fen
18	Germany	Abesinien, P-2	7	9	ombrotrophic bog
19	Finland	Kikilla	7	[e]absent	eutrophic fen
20	Estonia	West Estonia	7	absent	fen-bog transition

[a]degree of humification by von Post (1924).
[b]determined according to Heikurainen and Huikari (1952): 1) *S. angustifolium;* 2) *S. balticum;* 3) *S. capillifolium;* 4) *S. centrale;* 5) *S. compactum;* 6) *S. cuspidatum;* 7) *S. fallax;* 8) *S. fuscum;* 9) *S. imbricatum;* 10) *S. magellanicum;* 11) *S. molle;* 12) *S. palustre;* 13) *S. papillosum;* 14) *S. riparium;* 15) *S. russowii;* 16) *S. subsecunda.*
[c]according to Stewart and Kantrud (1971).
[d]*oligotrophic peat type.*
[e]*Sphagnum species was not found in this peat rather it was dominated by Carex* species.

2.3.2. Samples preparation and incubation

Microbial activity and biomass from 20 horticultural peat samples were determined by the SIR, FI, FE and AA methods and their relationships with a range of physicochemical characteristics were examined. Since water filled pore space (WFPS) measures the influence of moisture on microbial activity (Robertson and Groffman, 2007), the water content of each peat sample was adjusted to 40% of the corresponding WFPS with distilled and deionized water. These samples were conditioned for 10 d at 20°C (Rumed®, Rubarth Apparate GmbH, Germany) and subsequently used in the following methods.

2.3.2.1. Basal respiration

Basal/native respiration was measured without C and N amendments. Briefly, triplicate moist samples (10 g oven dry weight each) were incubated in separate 1.5 L glass jars for 10 d at 25 °C. The water content of the sample was adjusted to 60% of the corresponding water filled pore space (WFPS) with distilled and deionized water. The headspace gas was taken at the beginning and after 1, 2, 3, 4, 6, 8, and 10 d. After each headspace sampling, ambient gas was injected back into the jar to return the jar to its original pressure. Since the jar is a closed system, the volume of ambient gas injected is given by

$$V_i = \frac{C}{100} \times V_F \tag{1}$$

where V_i is volume of injected ambient gas (mL), C is the desired percentage of jar headspace volume to be collected and V_F is the headspace volume (mL). V_F was determined gravimetrically by subtracting the weight of jar containing sample from the weight of this jar when filled with water.

The concentrations of CO_2 were determined by gas chromatograph (Perkin Elmer Autosystem XL, Country) equipped with thermal conductivity detector (TCD). The oven was heated at 35 °C and helium was served as a carrier gas. The GC was calibrated with external standards of CO_2 (200, 5000, 10000 and 20000 μL) and showed a deviation of less than 5% for multiple injections. Peak area for CO_2 was determined by electronic integration and conversion of this area to μL CO_2 (V_{CO_2}) was made by measuring standard gases with the known CO_2 concentrations (200, 5000, 10000 and 20000 ppm). The concentration of CO_2 evolved over time was calculated using ideal gas law:

$$CO_{2t} = \frac{P \times V_{CO_2} \times 43.98983\ g\ CO_2\ mol^{-1}}{R \times T \times M_S} \tag{2}$$

where CO_{2t} (μg CO_2 g^{-1}) is the concentration of CO_2 at time t, (V_{CO_2}) is volume of CO_2 in the flask (μL) , P is pressure in kPa, R is the universal gas constant (8.31451 L kPa mol^{-1} K^{-1}), T is temperature in K and M_S is the oven-dry weight (odw) of incubated peat sample after drying it at 105 °C for 48 h.

2.3.2.2. Substrate-induced respiration (SIR)

Microbial biomass by the SIR method was estimated according to Anderson and Domsch (1978) as modified by West and Sparling (1986). Briefly, glucose was added to 10 g moist sample (n = 3) to achieve the peat-to-solution ratio of 1:2 (w/v). The final concentration in the sample was adjusted to 30 mg glucose mL^{-1}. Jars containing samples (each 250 ml capacity) were closed ≈15 min after glucose addition and incubated at 22 °C. Headspace gas was sampled at 0, 1, 2, 3, 4, 5, and 6 h after the jars were sealed. Following each headspace sampling, an equivalent volume of ambient air was injected back into the closed glass jar to avoid a drop in pressure. Correction was also made for dilution in the calculation of CO_2. Microbial biomass by the SIR method ($MB\text{-}C_{SIR}$) was computed from the maximum initial respiratory response (Anderson and Domsch, 1978) as this respiration rate best explains the size of the original microbial population. Microbial biomass-C was calculated according to the revised equation of Sparling et al. (1990) where $MB\text{-}C_{SIR}$ (µg g^{-1} dry peat) = 50 x (µl CO_2 g^{-1} dry peat h^{-1}).

2.3.2.3. Fumigation incubation (FI)

Subsamples were fumigated in a large desiccator with chloroform ($CHCl_3$) according to Jenkinson and Powlson (1976). After 24 h, the fumigant beaker was taken out and the residual $CHCl_3$ vapour removed by repeated evacuations (10-12 times) until the smell disappeared from the samples. Triplicate subsamples (each equivalent to 10 g odw) were weighed into 1.5 L capacity glass jars, inoculated with the respective 2 g odw unfumigated samples, and then incubated at 25 °C. The headspace gas was taken at the beginning (0 d) and end of incubation period (10 d) and analysed for CO_2. Similarly, conditioned and unfumigated samples were incubated in triplicate to serve as respective control treatments. CO_2 measurements from these control jars were taken at day 0 and 10. All jars were then opened, flushed with ambient air (for ca. 15 min) and incubated for additional 10 d. Microbial biomass-C ($MB\text{-}C_{FI}$) was calculated as: [(CO_2-C from fumigated samples (0-10 d) minus CO_2-C from unfumigated samples (10-20 d))/ proportionality factor (k_C)]. Here, we used a k_C of 0.45 (Vance et al., 1987a).

2.3.2.4. Fumigation extraction (FE)

FE was performed according to Vance et al. (1987b) with a modification on the extractant-to-peat ratio. From each peat type, triplicate subsamples (equivalent to 5 g odw each) were extracted with 0.5 M K_2SO_4 (1 h shaking at 80 rev min^{-1}) while the other three subsamples fumigated with chloroform ($CHCl_3$). In this study, a dry peat-to-extractant ratio of 1:25 (or ≈1:4 fresh sample at 40% WFPS-to-extractant ratio) was used as these peats had higher organic matter content and water holding capacity (Amha et al., 2010). In soils with large contents of organic and microbial materials, Scholle et al. (1992) also advised the use of a large extractant-to-soil ratio to improve results from the FE method. The volume of the filtrates (Schleicher & Schuell 595 ½), however, differed considerably between peat samples and tended to increase with increasing humification degree. After 24 h, $CHCl_3$ vapour from the fumigated samples was removed and subsequently extracted with 0.5 M K_2SO_4. The filtrates were analysed for total organic C (TOC) using a Shimadzu TOC analyzer (Shimadzu Corp., Kyoto, Japan). A 40-µL of these extracts was injected into the detection chamber for the analysis of TOC. The measured TOC between the repeated measurements of the same extract (n = 3) had ≥95% similarity, showing the reproducibility of the method (device). The increased extractable TOC due to fumigation (E_C; TOC from fumigated sample minus TOC from unfumigated samples) divided by the proportionality factor (k_{EC} = 0.45; Vance et al., 1987b; Joergensen, 1996) was considered to be C held in the microbial biomass ($MB\text{-}C_{FE}$; µg g^{-1} dry peat).

2.3.2.5. Arginine ammonification (AA)

AA was measured by the method of Alef and Kleiner (1987) with some modifications. Conditioned samples (equivalent to 5 g odw each) were weighed into six 250 mL plastic bottles and amended with 2.5 mL of 0.1% (w/v) L-arginine solution (Merck, Darmstadt, Germany). Three of these bottles were immediately frozen at –20 °C to serve as control treatment. The remaining three plastic bottles were placed inside 1.5 L glass jars, sealed with clip and incubated at 22 °C. After 3 h, the headspace gas was analyzed for CO_2 concentration while the peat samples were frozen overnight. All samples were then thawed in hot water, shaken with 2 M KCl (30 min, 80 rpm) and the filtrates were analysed for ammonium-N (NH_4^+–N) and nitrate-N (NO_3^-–N) using

Autoanalyzer (Alpkem Corp., Origen, USA). The rates of arginine mineralization (µg NH_4^+–N g^{-1} dry peat h^{-1}) were determined following Lin and Brookes (1999).

2.3.2.6. N-stability test

This test was conducted as described by VDLUFA (2006). Briefly, the pH of all peats was adjusted to 5.5–6.5 units by adding 1.25 g of 99.99% $CaCO_3$ (Merck, Darmstadt, Germany) per liter of peat. The contribution of ammonia volatilization at these chosen sample pH is minimal (Amha, 2006; Yosef and Bohne, 2009). Subsamples (each equivalent to m_B in eq. 1) were weighed into six 1000 ml plastic bottles and amended with 1 g N (as NH_4NO_3) and 0.05 g P (as K_2HPO_4) per liter dry peat basis. The sample moisture content was then adjusted to 80% of the respective maximum water capacity with distilled and deionized water. The final weight of incubated materials (m_B plus fertilizer solution plus distilled water) was 75 g.

$$m_B = \frac{75 \times D_{BD}}{(D_{BD} + 0.8 \times Vw_f) \times DM} \quad (1)$$

Where, m_B, D_{BD}, Vw_f and DM are the fresh weight of conditioned peat sample, dry bulk density, fresh bulk density at water capacity and dry matter percentage, respectively. Dry matter was computed after drying the conditioned peat sample over night at 105 °C while the water capacity (% v/v) determined using the quick method (Bohne and Wrede, 2004). Three plastic bottles from each peat type were then extracted immediately with CAT solution (1:8 w/v) and the other three were extracted after 2 weeks of incubation period (25°C). Both filtrates were analyzed for mineral N (N_{min}; NO_3^-–N + NH_4^+–N). N_{min} change in the samples was calculated by subtracting recovered N_{min} at week zero from N_{min} at week 2 (VDLUFA, 2006).

2.3.3. Calculations and statistical analyses

Each value in the tables and figures is the mean of three replicates. The extractable part of microbial biomass-C (k_{EC}) in each peat sample was computed by indirect calibration using the SIR method (Sparling et al., 1990). However, the overall k_{EC} factor was calculated from the reciprocal of the slope of the linear regression line that constrained to pass through the origin. The mean ratio of basal respiration-to-MB-C_{SIR} was taken as specific respiratory quotient (qCO_2, mg CO_2 g^{-1} MB-C d^{-1}). The Pearson correlation

coefficients between the microbiological data and a wide range of physicochemical parameters were computed using SAS (SAS Instit., Cary, NC).

2.4. Results

Basal respiration (evolution of CO_2 from unfumugated samples over 10 d; UF_{0-10}; Table 2) ranged from 24 to 128 µg CO_2-C g^{-1} dry peat d^{-1}, and was affected by species composition and the peat-forming environment. Peats dominated by *S. imbricatum* (9, 11, 14, 17 and 18) tended to produce the lowest basal respiration (36–62 µg CO_2-C g^{-1} dry peat d^{-1}) compared to the overall mean value of 77 µg CO_2-C g^{-1} dry peat d^{-1} (n = 20). With regard to the trophic status, the highest CO_2-C evolution was measured from eutrophic peats and the lowest from mesotrophic peats (except peat 15). Evolved CO_2-C from oligotrophic and transitional peats was quite variable.

The response of microorganisms to glucose addition varied considerably between peats of different origins. While the rates of CO_2 evolution (µL CO_2 g^{-1} dry peat h^{-1}) in the Lithuanian (n = 3) and Latvian (n = 2) peats decreased over 3 and 5 h, respectively, it was increased after 1 h in the Estonian (n = 2), Finish (n = 5), German (n = 2) and Swedish (n = 1) peat samples. However, the concentration of CO_2 was nearly stable over the incubation period in the Irish (n = 2), Estonian (peat 20) and the two German peats (peat 17 and 18). The maximum initial respiratory response in the tested peats (n = 20) ranged from 5.52 to 36.04 µL CO_2 g^{-1} dry peat h^{-1}, which corresponded to 276 to 1802 µg MB-C g^{-1} dry peat. In contrast, the amount of C held in the microbial biomass ($MB\text{-}C_{FE}$) ranged from 397 to 2172 µg g^{-1} dry peat (Table 2). In both cases, the maximum MB-C was obtained from peat 7 and the minimum MB-C from peat 20.

Evolution of CO_2-C from 6 fumigated peat samples was apparently lower than the corresponding unfumigated samples and resulted in a negative microbial biomass-C ($MB\text{-}C_{FI}$) (Table 2). When evolved CO_2-C from the unfumigated peat sample (measured between day 10 and 20; UF_{10-20}; Table 2) was used as a respective control treatment, all peat samples rendered positive flush-C ($MB\text{-}C_{FI}$ of 170 to 2411 µg g^{-1} dry peat; mean = 673 µg g^{-1} dry peat). However, the absolute values are mostly lower than the corresponding $MB\text{-}C_{SIR}$ and $MB\text{-}C_{FE}$.

Table 2

Microbial activity and biomass measured from a wide range of horticultural peats using the substrate-induced respiration (SIR), fumigation extraction (FE), fumigation incubation (FI), arginine ammonification (AA) and N-stability methods. Basal respiration is given as UF_{0-10}

peat samples	CO_2-C (µg g^{-1} dry peat d^{-1}) [a]UF_{0-10}	[b]UF_{10-20}	[c]F_{0-10}	MB-C (µg g^{-1} dry peat) SIR	FE	[d]FI	[e]AA	[f]N_{min} change (mg L^{-1})	[g]qCO_2	[h]k_{EC} factor
1[O]	70	64	106	739	609	935	73	−12.6	295	0.466
2[O]	73	66	72	983	924	224*	60	−25.2	347	0.593
3[T]	63	53	58	669	779	194*	53	−47.4	217	0.328
4[O]	102	51	160	1464	1592	2411	58	−13.9	351	0.373
5[O]	111	85	115	1091	1416	670	18	−72.5	341	0.423
6[T]	102	102	143	1798	1527	896	29	−35.9	257	0.455
7[O]	103	92	125	1802	2172	732	28	−75.8	235	0.284
8[O]	91	68	114	990	919	1020	66	−67.7	272	0.423
9[M]	36	30	45	517	523	342	56	−52.8	346	0.525
10[T]	76	76	81	1236	908	170	52	−38.2	227	0.331
11[O]	38	20	67	586	400	1044	54	−59.7	241	0.309
12[T]	73	66	71	1069	616	344*	58	−53.5	287	0.320
13[O]	82	65	88	1279	803	530	57	−104.6	254	0.263
14[T]	62	47	55	1058	766	177*	76	−59.9	255	0.491
15[M]	107	70	105	1368	974	768*	44	−44.3	299	0.561
16[E]	128	92	110	1653	1448	388*	56	−26.6	286	0.396
17[M]	37	36	55	393	518	430	63	−52.5	372	0.584
18[O]	41	33	50	515	532	381	70	−6.1	327	0.673
19[E]	113	65	121	1384	1741	1257	49	−124.5	208	0.382
20[T]	24	21	29	276	397	215	86	−61.4	209	0.542
Mean	**77**	**60**	**89**	**1044**	**978**	**673**	**55**	**−51.8**	**281**	**0.436**
CV (%)	**40**	**39**	**40**	**44**	**51**	**81**	**30**	**−56.9**	**18**	**26.6**

[a]mean daily CO_2-C evolution from unfumigated samples (over the first 10 d).
[b]mean daily CO_2-C evolution from unfumigated samples (between 10 and 20 d).
[c]CO_2-C from fumigated samples (0-10 d).
[d][(F_{0-10} minus UF_{10-20})/0.45];
[e]arginine ammonification in µg NH_4- g^{-1} h^{-1}.
[O]oligotrophic (ombrotrophic) peat.
[T]transitional peat.
[f]mineral N at week 2 minus mineral N at week 0.
[g]respiratory quotient in mg CO_2 g^{-1} MB-C d^{-1}.
[h]proportionality factor = total organic carbon flush/MB-C_{SIR}.
*[(F_{0-10} minus UF_{0-10})/0.45] is negative.
See Table 1 for the description of peat samples.
[M]mesotrophic peat.
[E]eutrophic peat.

Microbial activity (measured in terms of liberated NH_4^+–N from the added arginine solution) differed considerably between horticultural peat samples. Mineralized NH_4^+–N (indicated as AA in Table 2) ranged from 18 to 86 µg g^{-1} dry peat h^{-1}, with a grand mean value of 55 µg g^{-1} dry peat h^{-1} (n = 20).

At the beginning of incubation, nearly all of the added fertilizer (1 g NH_4NO_3-N L^{-1} dry peat) was recovered (except in peat 5 and 20) to suggest that surface fixation was insignificant in the tested horticultural peats (data not shown). The initial N_{min} tended to decrease in all peat samples after 2 weeks of incubation period in the range of 6.1 to 124.5 mg N L^{-1} (Table 2).

Table 3
Pearson correlation coefficient (*r*) computed between microbiological data and some of the selected physicochemical properties

Variables	BR (µg CO_2 g^{-1} d^{-1})	$MB\text{-}C_{SIR}$ (µg C g^{-1})	$MB\text{-}C_{FE}$ (µg C g^{-1})	$MB\text{-}C_{FI}$ (µg C g^{-1})	N_{min} change (mg N L^{-1})	AA (µg NH_4-N g^{-1} h^{-1})
H	−0.30	−0.34	−0.28	−0.21	−0.27	0.48*
OM	0.01	0.03	−0.07	0.07	0.46	0.16
C	−0.66**	−0.56**	−0.54*	−0.10	−0.08	0.45*
N	−0.14	−0.10	0.01	−0.02	−0.40	0.27
C/N	−0.06	−0.15	−0.18	−0.01	0.43	0.02
N/P	−0.67**	−0.66**	−0.45*	−0.18	0.04	0.58**
C/P	−0.62**	−0.66**	−0.54*	−0.13	0.32	0.43
pH	−0.15	−0.09	−0.03	0.07	0.22	0.37
AA-CO_2	0.88†	0.89†	0.84†	0.32	−0.10	−0.45*
AA	−0.61**	−0.61**	−0.67**	−0.19	0.24	
N_{min} change	−0.16	−0.13	−0.23	0.08		
$MB\text{-}C_{FI}$	0.35	0.31	0.40			
$MB\text{-}C_{FE}$	0.83†	0.86†				
$MB\text{-}C_{SIR}$	0.89†					

H–humification degree.
OM–organic matter.
AA-CO_2–mean evolved CO_2 from arginine treated samples (µg CO_2 g^{-1} dry peat h^{-1}).
BR–basal respiration.
N_{min} change–recovered mineral N at week 2 minus recovered mineral N at week 0.
*, **, *** or † donates significant level at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ or $P < 0.0001$ respectively.

The computed Pearson correlation coefficients between microbiological and the selected physicochemical data are presented in Table 3. Basal respiration showed poor correlations with the C/N ratio (r = −0.06), pH (r = −0.15), organic matter content (r = 0.01), the von Post humification degree (r = −0.30) and total N (r = −0.14) but correlated

relatively better with total C (*r* = –0.66). If the two eutrophic peats, which exceptionally showed the highest evolution rates, were excluded from the analyses, the respective *r-values* would have been improved for all variables. It appeared that evolved CO_2-C from unamended samples (basal respiration) explains about 69 and 79% of the total variation in MB-C_{FE} and MB-C_{SIR}, respectively (Table 3).

As it was a case for basal respiration, MB-C_{SIR} and MB-C_{FE} showed significant ($P < 0.05$) correlations with %C, the N/P and C/P ratio but correlated poorly with %N, pH, organic matter content, the C/N ratio and the von Post humification degree. However, both FI and AA methods showed poor correlations with basal respiration, MB-C_{FE}, MB-C_{SIR} and other physicochemical data (Table 3). The highest rates of AA were computed mostly for peats with less basal respiration, as confirmed by the computed negative *r*-value ($r = -0.61$; $P < 0.01$). The N_{min} change seems to have weak correlations with microbial activity, biomass, and all measured/computed physicochemical parameters (Table 3).

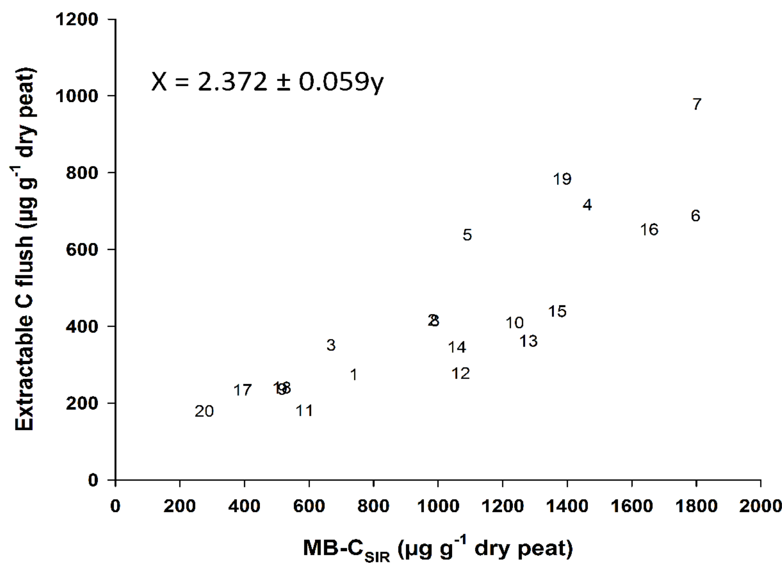

Fig. 1. Microbial biomass-C estimated by the SIR method as explained by the mean extractable C flush due to fumigation (E_C). The original regression line (x = 2.383y ± 5.623) has an intercept not significantly different from zero. Therefore, the equation: x = (2.372 ± 0.059)y was recalculated with constrain to pass through the origin. See Table 1 for peat numbering.

The regression line: MB-C = 2.372 ± 0.059 E_C gave an overall k_{EC} of 0.42 (Fig. 1), which was slightly lower than the mean value of individual k_{EC} computed as the ratio of E_C-to-MB-C_{SIR} (0.436; range = 0.263–0.673; Table 3).

2.5. Discussion

The rate of basal respiration is often used as a measure of microbial activity in diverse soils (Martens, 1987). Evolved CO_2-C from the eutrophic, oligotrophic, transitional and mesotrophic peats followed a descending order (120, 79, 67 and 60 CO_2-C µg g^{-1} dry peat d^{-1}, respectively) suggesting the presence of greater variations in the rates of C-cycling between these peat-forming environments. The higher microbial respiration in the eutrophic peats and the lower in mesotrophic peats may also suggest that microorganisms in the former trophic status were not constrained by the availability of easily degradable C sources. According to Shotyk (1988) and Steinmann and Shotyk (1997), the eutrophic peat is generally rich in nutrient as the aboveground biomass in the natural productive site decomposed regularly and renders easily decomposable plant materials. Less lignin content in ombrotrophic bog peats than that of the mesotrophic fens (Bohlin et al., 1989) might be one of the reasons for higher CO_2-C evolution in oligotrophic peats, as lignin is a less decomposable organic compound by microorganisms. Basal respiration in the transitional peat types varied widely (Table 2). These peats were obtained from areas where fens are turned to bogs.

The botanical composition of peat is also known to affect the rate of organic matter decomposition by microorganisms (Bohlin et al., 1989) as it determines the chemical nature of organic matter inputs in the peatlands. Such variations in the compositions of organic compounds could, thus, have effect on the rates of microbial activity in peat samples. Most peats in Table 1 composed of two or more *Sphagnum* species, and *S. fuscum* was abundantly found in the oligotrophic peats as it is one of the most common hummocks-growing *Sphagnum* species (Gorham, 1991). The minimum rates of CO_2-C evolution were measured from peats containing *S. imbricatum* (Table 2) to suggest that the organic compounds in these peats are relatively resistant to microbial degradation.

In the SIR method, emphasis is given to physiologically more active part of the total microbial biomass responding with larger respiration rate to the added C-source (Anderson and Domsch, 1978). The maximum initial respiratory responses in half of the tested peat samples were attained after 1 h. In contrast, the initial maximum respiratory

responses by the Latvian and Lithuanian peats were achieved after 3 and 5 h, respectively (data not shown), to suggest that the added glucose might have played a temporary inhibitory role in these peat samples. Microbial activity was expected to increase after the addition of easily degradable C substrate, but the decreases in the rates of evolved CO_2 in these peat samples were not expected. In their work, Anderson and Domsch (1978) have also observed a decrease in the rate of CO_2 over the first few hours for soils with high organic matter content, large biomass, and relatively high moisture level. Since all peat samples had high organic matter and moisture contents (Amha et al., 2010), factor(s) other than these may have been played a role in the Latvian and Lithuanian peats. The concentration of glucose solution used in this study was, however, much lower than the amount known to inhibit microbial respiration (i.e., 60 mg glucose mL^{-1} soil solution by West and Sparling (1986) and above 71 mg glucose mL^{-1} soil solution by Lin and Brookes (1999). A constant rate in 5 peat samples confirming both the activity and number of physiologically active microorganisms in these peat samples remain unchanged over the incubation period (6 h).

The computed MB-C_{FE} in this study varied between peat samples (397–2172 μg g^{-1} dry peat; Table 2) and was in similar range to that of Williams and Silcock (1997) (380–2170 μg C g^{-1} peat). However, the results obtained here were mostly lower than Croft et al. (2001) who measured wider ranges of MB-C_{FE} (1377–13873 μg g^{-1} peat) from natural, vacuum extracted and restored peatlands. It has been reported that the percentage of microbial biomass to total organic carbon was much lower in peatlands (0.56%) compared to agricultural forest soils (2.4%) (Moore and Basiliko, 2006). In this study, MB-C_{FE} comprised only 0.08 to 0.46% of the total C to indicate that the larger proportion of organic matter in peats is found in form of plant materials and remnants of humification processes. In general, peats with the highest basal respiration contained the highest MB-CFE (Table 2).

Mineralization of microbial biomass-C into CO_2 ($F_{0\text{-}10}$–$UF_{0\text{-}10}$)/k_C) is a base to estimate MB-C_{FI} (Jenkinson and Powlson, 1976), and it yielded negative biomass in 6 peat samples and very small flushes in the remaining ones (Table 2) to indicate that fumigation of peat samples leads to poor mineralization of microbial C over 10 d. In literatures, negative F_C values were also reported for highly organic arable soils

(Brookes et al., 1985) and strongly acidic (pH ~4.5) forest soils (Vance et al., 1987a). These authors summarized that the negative F_c might be caused by: (i) the inability of recolonizing population to metabolize non-microbial organic matter as fast as the native population in the unfumigated samples and/or (ii) the use of inadequate inoculums after fumigation. The second reason might not be valid here as we used 20% inoculums, which is optimal for acidic and organic soils (Vance et al., 1987a). Microbial biomass can also be estimated from evolved CO_2-C in fumigated sample without subtracting CO_2-C in the unfumigated although these results are prone to overestimation. Positive MB-C_{FI} in all peat samples were, however, obtained when evolved CO_2-C between day 10 and 20 (UF_{10-20}; Table 2) used as respective control samples. Overall, the biomass data computed in all cases had poor correlations with basal respiration, MB-C_{SIR} and MB-C_{FE} to confirm the widely accepted fact that the FI method is unsuitable to estimate biomass-C in wide range of horticultural peats, as this method was initially developed for well drained agricultural soils with a relatively low organic matter and near neutral pH (Sparling et al., 1990; Inubushi et al., 1991).

Amendment of soils with arginine resulted in immediate liberation of ammonia by living microorganisms (Alef and Kleiner, 1987; Andersen et al., 2006; Lin and Brookes, 1999), which can in turn be used to estimate microbial activity and biomass in soils. The rates of ammonification might, however, depend on the types of soil used. Alef and Kleiner (1987), for instance, reported AA of 0.51 to 13 µg NH_4-N g^{-1} dry soil h^{-1} for 34 investigated soils while Lin and Brookes (1999) measured from 0.1 to 17.1 µg NH_4-N g^{-1} dry soil h^{-1} (n = 13 soils). AA rates from the aerobic and anaerobic layers of natural, cutover and restored peatlands were also reported to be 17.1–22.5 and 16.8–22.0 µg NH_4-N g^{-1} dry peat h^{-1}, respectively (Andersen et al., 2006). The rates of ammonification in the current study (Table 2) were, however, substantially higher than the values mentioned above. This might be, among others, attributed to differences in samples handling and equilibration. Overall, AA showed poor correlations with other methods (Table 3) although close relationships (r = 0.83 to 0.91) between the rates of AA and microbial biomass (measured by FE, ATP content and SIR) were reported for mineral soils (Alef and Kliener, 1987; Lin and Brookes, 1999). Instead of liberated NH_4-N, evolved CO_2 from the arginine amended peats explained much of the total variations in the basal respiration, MB-C_{SIR} and MB-$C_{FE,}$ MB-C_{FI} measurements (Table 3).

When the MB-C_{SIR} data plotted against the K_2SO_4 extractable TOC flush, the later variable accounted for 26.2–67.3% of the microbial biomass-C (Fig. 1). Similar results have also been reported by Sparling et al. (1990) for 7 organic soils. Moreover, our computed mean k_{EC} (0.436) was nearly similar to the widely recommended value of 0.45 (Sparling et al., 1990; Joergensen, 1996) indicating the reliability of FE methods for measuring MB-C in a wide range of peat samples. MB-C_{FE} correlated strongly (r = 0.85) with MB-C_{SIR} to support the findings of Sparling et al. (1990) who reported a strong positive correlation (r = 0.89) in highly organic peat soils.

Despite the lowest *r*-value (Table 3), peats with the highest C contents (H7) were remained more stable than the lowest C containing peats (H3) to confirm that the organic C in the highly decomposed peats is the recalcitrant type (Buttler et al., 1994). Prasad and O'Shea (1997) have also reported such an inverse relationship between the basal respiration and the von Post humification scale. It should, however, be understood that the determination of humosity grade involves subjective evaluations that might produce data with low to moderate repeatability. The lack of any detectable pattern between the basal respiration and organic matter content or the C/N ratio indicating that microbial activity in *Sphagnum*-derived peat samples may not be predicted from these parameters. Although a higher *r* value was computed between basal respiration and total C (Table 3), the K_2SO_4 extractable TOC explained much of the variation in native respiration to suggest that organic C separation by different extractants (e.g. cold water, hot water, salt) might produce a simple and persuasive indicator behind the measured rates of C-cycling in the peat samples.

N-stability test is designed to give a quality assurance for growers on the stability of the N-balance (VDLUFA, 2006), where an N_{min} change of <50, 50–125 or >125 mg N L^{-1} characterized as stable, less stable or unstable potting media, respectively. These classifications may not, however, reflect the behaviour of potting media as the method ignores important conditions exhibited during plant cultivation (e.g. availability of easily degradable C). The conventional wisdom in the cultivation of potted plants indicating that the whole media turned very quickly to rhizosphere and the availability of easily decomposable C sources for microorganisms increased dramatically. Moreover, the

higher moisture content (i.e., 80% of the respective maximum water capacity) may tend to favor denitrification. This was confirmed by a separate experiment where the rates of denitrification from peats were considerably high at ~80% of the respective water capacity (Amha and Bohne, 2011). N_{min} change over 2 weeks (N_{min} change = recovered N_{min} at week 2 minus recovered N_{min} at week 0) portrayed weak relationships with the other tested methods to cast doubt on the effectiveness of the N-stability test in controlling the quality of potting media. There also appeared that no trends can be drawn between the tested peat types (Table 2). It is, therefore, of a paramount importance to reconsider the entire incubation conditions (mainly the moisture content, sample size, N_{min} amount and addition of easily decomposable C source) to maximize the effectiveness of this method in explaining the N-balance of the potting media. The use of ^{15}N might also help to identify the actual N-transformations activity in the potting media rather than just relying on a mass balance calculation (VDLUFA, 2006).

2.6. Conclusion

Evolved CO_2 from unamended peat (basal respiration) explains about 69 and 79% of the total variation in MB-C_{FE} and MB-C_{SIR}, respectively. The computed k_{EC} value (0.436 ± 0.09) was nearly similar to the widely accepted value for soils (0.45; Sparling et al., 1990; Joergensen, 1996) to confirm that FE and SIR can be used as reliable methods to estimate microbial biomass-C in a wide range of peat samples. However, this was not the case for AA and FI methods. Microbial activity in the eutrophic peats was considerably high regardless of their respective botanical composition and degree of humification to suggest that these might not maintain their structural stability during crop cultivation. The higher microbial activity in these peats might also induce competition for nutrients/air between plant root and microorganisms. Basal respiration, MB-C_{SIR} and MB-C_{FE} in the tested peat samples were poorly predicted by H, %C, %N, pH, OM content, and the C/N ratio. Therefore, the second experiment was designed to fractionate the total OM into different components so as to get a simple component that acceptably predicts microbiological properties of peats.

3. Water- and salt-extractable organic carbon from peats: their relations with long-term CO_2 evolution, microbial biomass-C, and the degree of decomposition

3.1. Abstract

The amounts of water- and salt (0.5 M K_2SO_4)-extractable organic carbon (OC) components, and their relations with the von Post degree of humification (H), peat-forming environment, microbial activity and biomass-C were studied using a range of peat samples with contrasting properties. The microbial activity was measured as evolved CO_2 over six months. Total OC (TOC) in the tested peat samples (n = 20) ranged from 469 to 522 mg g^{-1} dry peat with an overall mean value of 496 mg g^{-1} dry peat. The amounts of dissolved OC (µg C g^{-1} dry peat) were increased from cold-water extractable OC (CWC; 57 to 199; mean = 118) to salt-extractable OC in the hot-water bath (SSC-H; 628 to 3335; 1847) through salt-soluble OC (SSC; 342 to 1061; 691) and hot-water extractable OC (HWC; 331 to 3118; 1414). The eutrophic peats contained the highest mean dissolved OC followed by the oligotrophic, transitional and mesotrophic peat types. TOC measurements showed weak and negative correlations with the mean daily CO_2 evolution over six months (r = −0.62) and biomass-C (r = −0.55 to −0.57). Microbiological data, however, showed considerably higher and positive correlations with all dissolved organic fractions to suggest that extraction of dissolved OC would provide greater insight into the labile OM responsible to the degradation of peat samples. The evolved CO_2 from peat samples were poorly correlated with H (r = −0.17 to −0.34). Inclusion of independent variable such as TOC, CWC, HWC, SSC, SSC-H, or potentially mineralizable-N into a step-wise linear regression analysis significantly ($p < 0.05$) improved the relationships between H and microbiological data. Overall, HWC and SSC-H showed strong correlations with long-term evolved CO_2 (r = 0.94 and r = 0.92, respectively) and MB-C (r = 0.89−0.90 and r = 0.85−0.87). It should be noted, however, that salt extracted OC had less deviation within replications, indicating a higher reproducibility of SSS-H data than CWC.

Keywords: cold-water extractable OC (CWC), hot-water extractable OC (HWC), microbial activity and biomass, peat-forming environment, salt-extractable OC (SSC), salt-extractable OC in hot-water bath (SSC-H), total OC, von Post humification degree

3.2. Introduction

Peats in fens, bogs, and marshes contain 0.5 * 10^{11} Mg carbon (C) (Succow and Jeschke, 1990) indicating peats in the natural ecosystem serve as C sink, as the rates of decomposition are considerably low. However, some peat-forming environments (e.g. a temperate poor fen) have been identified to be potential net sources of CO_2 to the atmosphere (Caroll and Crill, 1997). Peat used as potting media can also be a source of C although the amount is not yet known. The rates of microbial degradation in peats/soils are dependent of several factors including the fraction of the intrinsic organic matter (OM) that heterotrophic microorganisms can easily use as carbon and energy sources (Davidson et al., 1987). Organic matter in peat, unlike mineral soil, represents up to 100% of the respective dry mass (Clymo, 1983). However, OM contents in peats had very poor correlation with the activity or biomass of microorganisms (Chapter 2). It can, thus, be hypothesized that extraction of the OM by water and salt could render a simple but persuasive indicator about the activity and biomass of microbes in peat samples.

Quantification of the readily decomposable OM is essential to predict the rate of C mineralization (Davidson et al., 1987). Wagai and Sollins (2002) suggested that the water-soluble OM in soils is an easily biodegradable organic carbon (OC) because of its small molecular size and its accessibility to microbes. Fischer (1993), on the other hand, reported that the hot-water extractable organic C (HWC) contents in soils (as compared to the total OC) were strongly correlated with CO_2 evolution, which would indicate that a proportion of the HWC must be easily available for microbial utilization. In this study, therefore, it was assumed that microbial activity and biomass of peats could be greatly influenced by water or salt extractable OC than the total OC. In line with this, peat with a higher amount of dissolved OC assumed to support a higher microbial activity than peat with a lower amount of dissolved OC.

Investigating the relationships between different OM components and microbiological data could provide some insight into the microbially mediated C and N transformations in peat samples. So far, however, there is a dearth of information about the quantitative range (size) of water- and salt-soluble organic components in peats that widely differ in

degree of humification, botanical composition, and peat-forming environments, and their relationships with the microbiological data. Therefore, the objectives of this particular study were (i) to extract and quantify different OM components from peat samples, and (ii) to examine the statistical associations between these components and microbiological properties (CO_2 production over six months and biomass-C). Moreover, attempts were made to improve the relationships between the most commonly used parameter to describe peat samples (i.e., the von Post humification degree) and the microbiological data by incorporating these OC fractionates in the step-wise multiple regression analyses.

3.3. Materials and Methods

3.3.1. Peat samples

Twenty peat samples were included in this study (Table 1). Each peat type was delivered to us in three separate plastic bags and each bag was considered as a replicate. Subsamples were sieved (<5 mm) and analyzed for total C and total N (Vario MAX CN analyzer, Hanau, Germany). The presence of carbonate (CO_3) in peats was checked by gas-volumetric analysis after applying 0.5 M phosphoric acid (H_3PO_4); and evolved CO_2 from all but peat 20 (0.5 mg CO_3-C g^{-1} dry peat) was insignificant. Hence, the total organic C in peat 20 was computed as the difference between total C and CO_3-C. The OM content, expressed as percentage of the initial oven dried sample weight (odw), was determined by dry combustion at 550 °C for ca. 16 h. The degree of humification was determined according to the von Post scale (von Post, 1924) by which an unhumified peat is designated as H1 and a completely humified (amorphous) peat as H10.

Table 1
Details on the peat-forming environment and botanical composition of peat samples obtained from seven European countries

Peat types	Country	[a]H	Organic matter (%)	N (%)	[b]Relevant *Sphagnum* species	[c]Peat-forming environment
1	Latvia	3	99.8 ± 0.1[d]	0.48 ± 0.00	2, 5, 8, 10	ombrotrophic bog[e]
2	Lithuania	3	99.2 ± 0.0	0.75 ± 0.00	4, 8, 11, 15	ombrotrophic bog
3	Lithuania	3	99.0 ± 0.1	0.71 ± 0.01	1, 6, 8, 10	fen-bog transition
4	Estonia	3	98.6 ± 0.0	0.95 ± 0.01	2, 6, 8	ombrotrophic bog
5	Finland	3	98.2 ± 0.2	0.74 ± 0.01	5, 8, 10, 15	ombrotrophic bog
6	Finland	3	98.7 ± 0.0	1.04 ± 0.01	1, 10	fen-bog transition
7	Finland	3	97.7 ± 0.3	0.86 ± 0.01	1, 8	ombrotrophic bog
8	Latvia	4	99.5 ± 0.0	0.93 ± 0.00	1, 6, 8, 10	ombrotrophic bog
9	Ireland	4	99.1 ± 0.1	0.76 ± 0.00	9	mesotrophic fen
10	Lithuania	4	97.8 ± 0.1	1.06 ± 0.00	1, 3, 10, 14	fen-bog transition
11	Ireland	4	98.6 ± 0.0	0.83 ± 0.01	1, 8, 9	ombrotrophic bog
12	Germany	4	96.9 ± 0.3	0.90 ± 0.00	8, 10, 13	fen-bog transition
13	Estonia	5	99.2 ± 0.1	0.88 ± 0.01	8	ombrotrophic bog
14	Sweden	5	95.9 ± 0.2	1.04 ± 0.01	8, 9	fen-bog transition
15	Germany	5	95.9 ± 0.0	1.02 ± 0.01	7, 13	mesotrophic fen
16	Finland	5	96.2 ± 0.4	0.87 ± 0.01	16	eutrophic fen
17	Germany	6	97.1 ± 0.0	1.02 ± 0.01	9, 12	mesotrophic fen
18	Germany	7	96.3 ± 0.1	1.05 ± 0.01	9	ombrotrophic bog
19	Finland	7	91.7 ± 0.6	1.65 ± 0.00	[f]Absent	eutrophic fen
20	Estonia	7	91.4 ± 0.2	2.08 ± 0.01	Absent	fen-bog transition

[a]degree of humification by von Post (1924).
[b]determined according to Heikurainen and Huikari (1952): 1) *S. angustifolium;* 2) *S. balticum;* 3) *S. capillifolium;* 4) *S. centrale;* 5) *S. compactum;* 6) *S. cuspidatum;* 7) *S. fallax;* 8) *S. fuscum;* 9) *S. imbricatum;* 10) *S. magellanicum;* 11) *S. molle;* 12) *S. palustre;* 13) *S. papillosum;* 14) *S. riparium;* 15) *S. russowii;* 16) *S. subsecund.a*
[c]according to Stewart and Kantrud (1971).
[d]mean ± standard deviation (n = 3).
[e]ombrothrophic peats are oligotrophic peats.
[f]*Sphagnum species was not found rather it contained Carex* species.

3.3.2. Organic C components

The total OM in peats were fractionated into (*i*) total OC (TOC), (*ii*) cold-water extractable OC (CWC), (*iii*) hot-water extractable OC (HWC), (*iv*) salt-extractable OC (SSC), and (*v*) salt-extractable organic C in hot bath (SSC-H) (Fig. 1). The CWC and SSC were removed using deionized water and 0.5 M K_2SO_4, respectively. Briefly, 5 g of subsample (odw) was shaken with 100 mL water or 0.5 M K_2SO_4 solution for 1 h at 80 rpm. Then, the supernatant was recovered by centrifuging (for 1 h at 3000 rpm and at 25 °C) and filtered through 0.45 μm cellulose nitrate membrane filters by applying suction. The HWC was also extracted with distilled and deionized water. Peat sample (5 g odw each, n = 3) was shaken with 100 mL distilled water for 2 min (to resuspend the peat samples) and left in hot-water bath (80 °C) for ~16 h using a modified method of

Haynes and Francis (1993). The bottles were capped while the samples are in the bath. The samples were centrifuged for 1 h (3000 rpm, 25 °C) and the supernatants were filtered through 0.45 μm cellulose nitrate membrane filters by applying suction. The SSC-H was extracted in similar way to that of HWC except 0.5 M K_2SO_4 solution was used as extractant. Liquid collected from the peat samples (CWC, HWC, SSC and SSC-H) were analyzed for dissolved OC using a Shimadzu TOC analyzer (Shimadzu Corp., Kyoto, Japan).

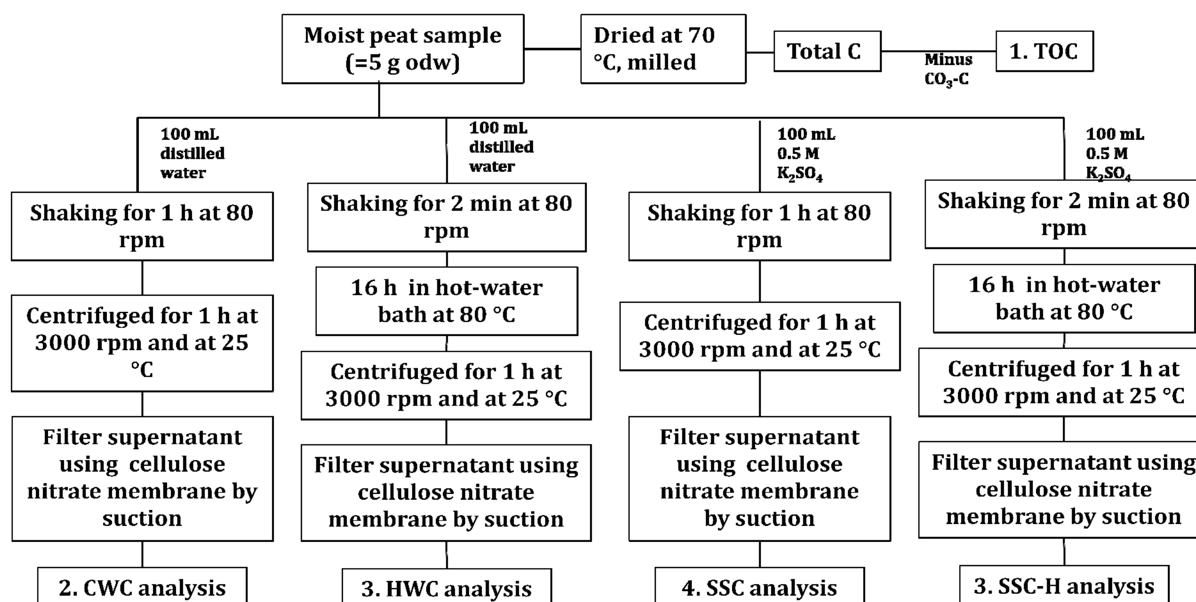

Fig. 1. Schematic presentation of organic C fractionations from the peat samples that differed widely in their humification degree on the von Post scale (H), floristic composition and the peat-forming environments. The five fractionated organic matter are: total organic carbon (TOC), Cold-water extractable OC (CWC), hot-water extractable OC (HWC), salt-extractable OC (SSC), salt-extractable OC in hot-water bath (SSC-H).

3.3.3. Microbial activity

Subsample from each bag and peat type was taken and its respective moisture content adjusted to 40% water filled pore space with distilled and deionized water, and conditioned for 10 d at 20 °C. Triplicate moist samples (equivalent to 150 mL each) from each peat type were packed into plastic pots (based on their respective bulk density; Amha et al., 2010). Each pot was then kept inside a 1.5-L glass jar and incubated at 25 °C. The hole on the cap was entirely covered by a rubber septum through which the sampling needle was pierced. Headspace gas (20 mL) was taken from each sealed jar at the beginning and 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, and 180 d. After each

sampling, the jars were opened, checked for moisture loss and incubated further. The concentrations of CO_2 in the headspace samples were measured by gas chromatograph (GC). The GC oven temperature was 35 °C and the carrier gas (He) was flowing at the rate of 30 mL min^{-1}. The mean daily emission from a given peat sample (µg CO_2 g^{-1} dry peat d^{-1}) was estimated using an equation developed from the measured CO_2 vs the incubation period.

3.3.4. Microbial biomass

Microbial biomass-C by fumigation extraction method (MB-C_{FE}) was estimated according to Vance et al. (1987b). Briefly, 5 g odw sample was extracted with 0.5 M K_2SO_4 (moist sample-to-solution ratio of 1:4 (w/v)) and filtered by Schleicher & Schuell 595 ½. The second portion was fumigated with ethanol free $CHCl_3$ for 24 h at room temperature. The $CHCl_3$ in the samples was then removed by subsequent evacuations (10-12 times) and extracted in the same way to that of the unfumigated samples. Both extracts were analyzed for dissolved OC as described above. The increases in the extractable C due to fumigation were taken as MB-C with the k_{EC} value of 0.45 (Joergensen, 1996; Sparling et al., 1990). Microbial biomass-C by the substrate-induced respiration method (MB-C_{SIR}) was assessed using the approach of Anderson and Domsch (1978) as modified by West and Sparling (1986). Triplicate moist samples (10 g each) were amended with glucose solution to achieve the peat-to-solution ratio of 1:2 (w/v). The final concentration in the sample solution was adjusted to 30 mg glucose mL^{-1}. Evolved CO_2 was measured on hourly basis over 6 h. MB-C_{SIR} was calculated using an equation given by Sparling et al. (1990).

3.3.5. Potentially Mineralizable N

Potentially mineralizable N (PMN) was measured anaerobically by Keeney and Bremner (1966) method. Triplicate subsamples (equivalent to 5 g odw each) were placed into 1000 mL plastic bottles with screw tops and 100 mL of deionized water was added to create a minimum suspension of 1 cm. After swirled the bottles for a few seconds, the slurries were incubated for two weeks at a constant temperature of 40 °C. Then, K_2SO_4 (equivalent to 0.5 M) was added, shaken for 1 h at 80 rpm and filtered using Schleicher &

Schuell 595 ½. Inorganic-N (i.e., the sum of NH_4^+–N and NO_3^-–N) was measured by autoanalyzer (Alpkem Corp., Origen, USA). Triplicate samples from each peat type were also extracted with K_2SO_4 at the beginning of incubation. The difference of inorganic-N measurements between incubated and non-incubated samples was considered as PMN.

3.3.6. Calculations and statistical analyses

Each value in the Tables and Figures is the mean of three separate measurements. The coefficient of variation (CV, %) was calculated as the ratio of the standard deviation to grand mean, as it is a useful statistic for comparing the degree of variation from one peat data series to another, even if the means are drastically different from each other. Correlation analysis (SAS Proc CORR) was used to detect relationships among microbial variables (CO_2 evolution and MB-C) and the amount of OM fractionates. Stepwise multiple regressions were also carried out by considering microbiological properties as the dependent variables and the other biochemical parameters as independent variables. Grouping of peat samples were performed using the OC fractionates (TOC, CWC, HWC, SSC, SSC-H), respiratory (CO_2 evolution), microbial biomass-C (by FE and SIR) and PMN data on the basis of hierarchical cluster analysis. The dendrograms were established using the Ward linkage method based on the Euclidean square distances.

3.4. Results

3.4.1. Initial characteristics

Peats in Table 1 were classified into four trophic levels: eutrophic (n = 2), mesotrophic (n = 3), oligotrophic (n = 9), and the transitional peats (n = 6). Likewise, these peats were broadly classified into (i) weakly humified/fibric (≤H3; n = 7), (ii) moderately humified/mesic (H4–H6; n = 10), and (iii) strongly humified/humic (≥H7; n = 3). The mean OM percentage in the strongly humified peats (93.1) was lower than the corresponding values in the moderately (97.6) and weakly (98.8) humified peats. The highest mean total N contents were measured mostly from the more humified peats and conversely the lowest from the less humified peats. All peat samples (except peat 19 and

20) are predominantly composed of *Sphagnum* moss species. Details in the physical and chemical properties of these peats were reported by Amha et al. (2010).

Table 2.
Organic carbon (OC) fractionates from a wide range of peat samples

Peat[a]	Extractable OC (μg g^{-1} dry peat)				
	Total	Cold-water	Hot-water	Salt-	SSC-H[b]
1[O]	48.0 × 10^4	122 ±13[c]	1206 ± 207	840 ± 28	1918 ± 16
2[O]	49.0 × 10^4	106 ± 6	1077 ± 157	619 ± 19	1580 ± 86
3[T]	49.0 × 10^4	98 ± 10	803 ± 126	562 ± 7	1245 ± 38
4[O]	49.1 × 10^4	113 ± 9	1892 ± 88	687 ± 21	2791 ± 94
5[O]	47.8 × 10^4	164 ± 12	2074 ± 81	942 ± 27	2244 ± 88
6[T]	49.3 × 10^4	106 ± 9	1892 ± 53	904 ± 11	2526 ± 50
7[O]	47.2 × 10^4	199 ± 10	2418 ± 198	1061 ± 53	2920 ± 84
8[O]	49.3 × 10^4	142 ± 12	905 ± 182	806 ± 50	1791 ± 49
9[M]	50.7 × 10^4	78 ± 7	794 ± 176	419 ± 13	917 ± 35
10[T]	50.0 × 10^4	90 ± 8	1853 ± 102	600 ± 15	2123 ± 44
11[O]	50.9 × 10^4	58 ± 5	636 ± 137	484 ± 8	726 ± 17
12[T]	50.5 × 10^4	128 ± 6	1435 ± 235	864 ± 26	1732 ± 63
13[O]	48.9 × 10^4	135 ± 7	1168 ± 133	661 ± 7	1429 ± 51
14[T]	51.2 × 10^4	132 ± 25	1174 ± 113	660 ± 20	1866± 76
15[M]	48.8 × 10^4	149 ± 20	1841 ± 177	759 ± 19	2333± 60
16[E]	46.9 × 10^4	172 ± 24	2592 ± 333	974 ± 45	2909± 33
17[M]	51.5 × 10^4	59 ± 10	504 ± 83	342 ± 17	827± 57
18[O]	52.2 × 10^4	58 ± 17	574 ± 106	351± 18	1093± 79
19[E]	51.2 × 10^4	186 ± 5	3118 ± 179	815 ± 8	3335± 33
20[T]	50.2 × 10^4	57 ± 14	331 ± 85	480 ± 19	628± 26
Mean	**49.6× 10^4**	**118**	**1414**	**691**	**1847**
CV(%)	**3 × 10^4**	**37**	**54**	**30**	**43**

[a]see Table 1 for description.
[b]salt-soluble organic C in hot-water bath.
[c]mean ± standard deviation (*n* = 3).
[E]eutrophic peat.
[O]oligotrophic (ombrotrophic) peat.
[M]mesotrophic peat.
[T]transitional peat.

3.4.2. Organic C fractions

Total organic C (TOC) in the peat samples ranged from 469 to 522 mg g^{-1} dry peat with an overall mean value of 496 mg g^{-1} dry peat (Table 2). The amounts of dissolved OC in all peat samples were increased from cold-water extractable OC (CWC) to salt-extractable OC in the hot-water bath (SSC-H) through salt-soluble OC (SSC) and hot-water extractable OC (HWC). The overall mean OC in SSC-H (1847 μg OC g^{-1} dry peat d^{-1}) were 15.7 times higher than that extracted by cold-water. The mean OC in SSC-H was also 131% of HWC, 263% of SSC, and 0.37% of TOC. The overall mean values of CWC,

HWC, SSC and SSC-H concentrations in the eutrophic peats (179, 2855, 894, and 3122, respectively) were higher than the corresponding values in the oligotrophic peats (121, 1328, 717, and 1881), which was followed by the concentrations in the transitional (102, 1247, 678, and 1614) and the mesotrophic peats (96, 1047, 507, and 1359). All dissolved OC in the water and salt extracts showed decreasing trends with increased H-levels when the two eutrophic peats are excluded from the analysis (Table 2).

Table 3.
Evolution of CO_2 over six months and microbial biomass estimated by fumigation extraction (MB-C_{FE}) and substrate induced respiration (MB-C_{SIR}) from a wide range of peat samples

Peat[a]	CO_2 (µg g dry peat $^{-1}$ d^{-1})	(µg C g^{-1} dry peat)	
		[b]MB-C_{SIR}	[b]MB-C_{FE}
1[O]	185 ± 9[c]	739 ± 69	609 ± 13
2[O]	267 ± 10	983 ± 46	924 ± 55
3[T]	201 ± 16	669 ± 47	779 ± 33
4[O]	275 ± 26	1464 ± 62	1592 ± 62
5[O]	330 ± 21	1091 ± 32	1416 ± 124
6[T]	313 ± 16	1798 ± 66	1527 ± 77
7[O]	362 ± 22	1802 ± 100	2172 ± 216
8[O]	194 ± 9	990 ± 123	919 ± 88
9[M]	90 ± 9	517 ± 24	523 ± 59
10[T]	273 ± 14	1236 ± 68	908 ± 68
11[O]	83 ± 4	586 ± 64	400 ± 12
12[T]	221 ± 13	1069 ± 139	616 ± 49
13[O]	233 ± 3	1279 ± 52	803 ± 95
14[T]	151 ± 6	1058 ± 72	766 ± 55
15[M]	300 ± 11	1368 ± 53	974 ± 48
16[E]	435 ± 23	1653 ± 70	1448 ± 179
17[M]	130 ± 7	393 ± 44	518 ± 59
18[O]	109 ± 11	515 ± 42	532 ± 61
19[E]	407 ± 14	1384 ± 78	1741 ± 121
20[T]	58 ± 2	276 ± 48	397 ± 61
Mean	**231**	**1044**	**977**
CV(%)	**47**	**44**	**51**

[a]see Table 1 for description.
[b]adopted from Table 2 of chapter 2.
[c]mean ± standard deviation (*n* = 3).
[E]eutrophic peat.
[O]oligotrophic (ombrotrophic) peat.
[M]mesotrophic peat.
[T]transitional peat.

3.4.3. Microbial respiration and biomass

The highest mean daily CO_2 evolution over six months was measured from peat 16 and 19 (both are the eutrophic peats) while the lowest CO_2 measured from peat 20 (Table 2). Mean daily respiration (± standard deviation) from the eutrophic, oligotrophic, transitional, and mesotrophic peats were 421 ± 20, 217 ± 97, 216 ± 87, and 173 ± 112

CO_2 µg g^{-1} dry peat d^{-1}, respectively. The highest standard deviations in all but eutrophic peats indicating that peats of the same trophic level differed considerably in their biological properties, which intern limit a general conclusion about the effect of peat-forming environment on the rates of microbial respiration. The exclusion of eutrophic peats from the analysis (because of their highest microbial respiration) would result in a relatively more stable strongly humified peats (83 ± 36 µg CO_2 g^{-1} dry peat d^{-1}) followed by the mean respiration from the moderately (186 ± 78 µg CO_2 g^{-1} dry peat d^{-1}) and the weakly humified peats (276 ± 65 µg CO_2 g^{-1} dry peat d^{-1}) to suggest that OC in the weakly humified peats is relatively less resistant to microbial degradation than peats in the other classes. Concerning the botanical composition, the minimum rates of CO_2 evolution were measured mostly from peats containing *Sphagnum imbricatum* species (peat 9, 11, 14, 17, and 18).

Microbial biomass-C by SIR and FE methods ranged from 276 to 1802 and from 397 to 2172 µg g^{-1} dry peat, respectively (Table 2). Clear trends between the degree of humification and the mean MB-C estimated by both methods were observed when the two-outlier data (i.e., the eutrophic peats) excluded from the calculations. It appeared that the weakly humified peats contained the highest MB-C_{SIR} and MB-C_{FE} (1221 and 1288 µg g^{-1} dry peat, respectively) whereas the strongly humified peats had the lowest (396 and 465 µg g^{-1} dry peat). The corresponding values in the moderately humified peats (H4–H6) were 944 and 714 µg g^{-1} dry peat. When peats were classified based on the peat-forming environments, the mean MB-C_{FE} data (µg g^{-1} dry peat) followed a similar ranking as of CO_2 evolution: mesotrophic < transition < oligotrophic < eutrophic peat type.

3.4.4. Relationships between OC fractionates and microbiological data

When each peat type represented by a single data point, the TOC measurements showed weak and negative correlations with microbial activity ($r = -0.62$), MB-C_{SIR} ($r = -0.57$) and MB-C_{FE} ($r = -0.55$) (Fig. 2b). However, the computed *r*-values were significantly improved by OC fractionation (Fig. 2c–f). It was found that microbial activity data (measured as mean daily CO_2 evolution over six months) correlated well with HWC ($r = 0.94$) followed by SSC-H ($r = 0.92$), CWC ($r = 0.82$) and SSC ($r = 0.79$) to suggest that these fractionates represented the amount of a readily mineralizable OC in peats than the TOC. However, microbial activity

and biomass-C were better explained by extracts from the hot-water bath (HWC and SSC-H) compared to extracts from cold-water or salt solutions (CWC and SSC).

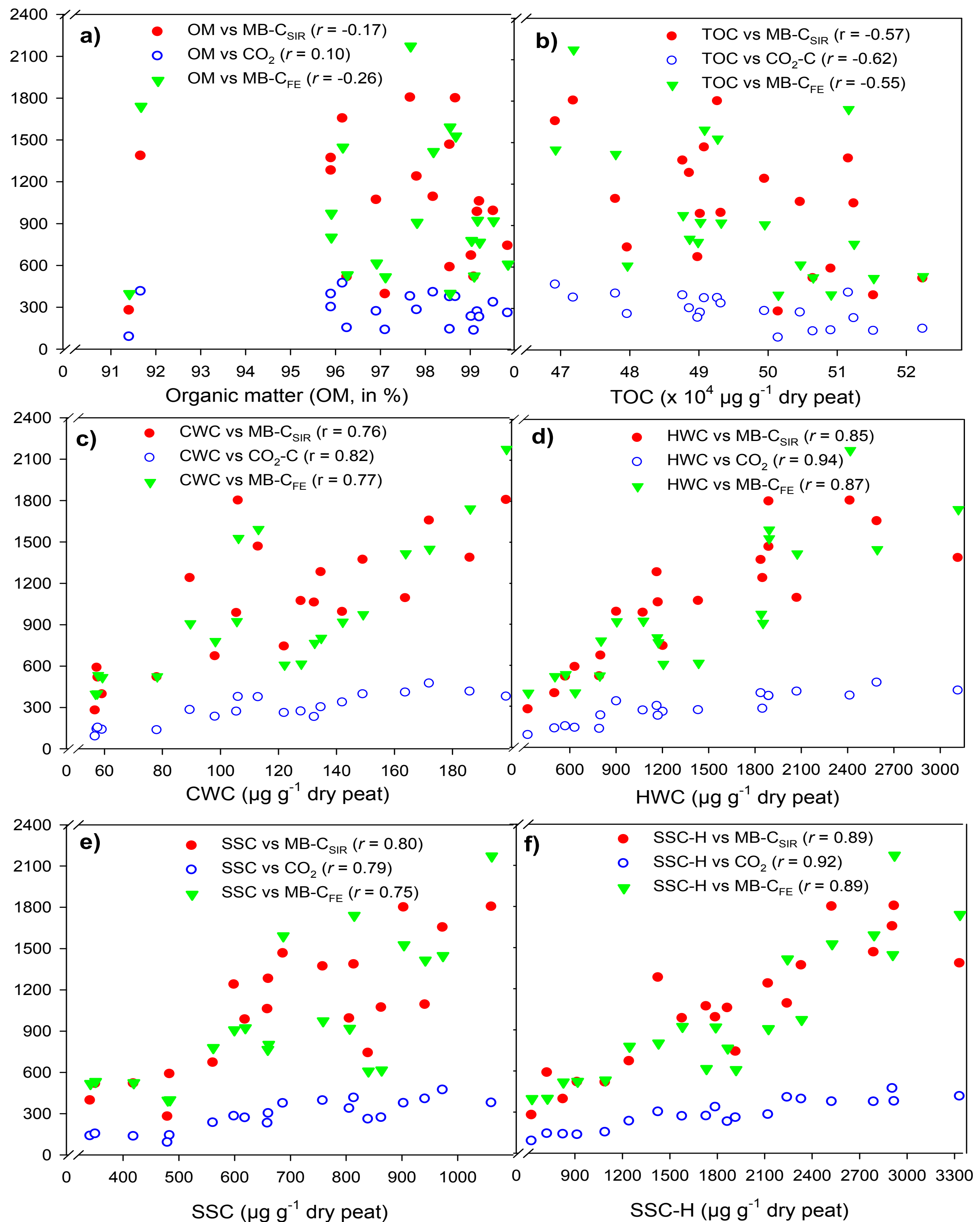

Fig. 2. The relationships of evolved CO_2 over six months (y-axis; µg g^{-1} dry peat d^{-1}) or microbial biomass-C (y-axis; µg g^{-1} dry peat) estimated by the fumigation extraction (MB-C$_{FE}$) and substrate-induced respiration (MB-C$_{SIR}$) methods with: a) organic matter (OM), b) total organic C (TOC), c) cold-water OC (CWC), d) hot-water OC (HWC), e) salt OC (SSC), and f) salt-extractable OC in hot-water bath (SSC-H).

The evolved CO_2, MB-C_{SIR} and MB-C_{FE} from peat samples were poorly correlated with H (r = −0.17 to −0.34; Table 3). The inverse relations, however, indicating that MB-C and evolved CO_2 per unit dry peat were lower in more decomposed peats (high H-value) than the weakly humified ones. The step-wise linear regression analyses have been performed to test whether the inclusion of OC fractionates into the model could improve the resulting correlations between H and microbiological data. It appeared that addition of independent variable such as TOC, CWC, HWC, SSC, SSC-H, or Potentially Mineralizable-N (PMN) into the equation significantly (P < 0.05) improved the relationships between H and microbiological data (Table 3). However, the inclusion of %N or OM contents into the model had no or little effect on the computed r^2-values.

Table 3.
Regression analyses between the degree of humification (H) and long-term microbial respiration (CO_2) or microbial biomass-C estimated by the substrate-induced respiration (MB-C_{SIR}) and fumigation extraction (MB-C_{FE}) methods. Increase in r^2 indicates the change in r^2 when a given peat property is included in a multiple linear regression model

	r^2 (%)	Increase in r^2 (%)							
	H	OM	TOC	SSC	CWC	HWC	SSC-H	%N	PMN
CO_2	2.9[a]	23.9	37.8*	54.2***	61.4†	75.6†	73.1†	1.4	62.9†
MB-C_{SIR}	11.4[a]	15.2	20.4*	52.8**	49.8†	66.0†	70.0†	4.4	40.5**
MB-C_{FE}	7.8[a]	23.4*	22.1*	48.0**	53.7†	70.4†	73.2†	9.4	25.7*

[a]the correlation (r) was negative.
OM – organic matter.
TOC – total organic C.
SSC – salt (0.5 M K_2SO_4) extractable organic C.
CWC- cold-water extractable organic C.
HWC – hot-water extractable organic C.
SSC-H – salt extracted organic C in hot-water bath.
PMN – potentially mineralizable N.
*, **, *** or † donates significant level at P ≤0.05, P≤0.01, P≤0.001 or P<0.0001 respectively.

Determination of MB-C_{FE} requires extraction of dissolved organic C by 0.5 M K_2SO_4 before (SSC) and after fumigation (SSC-F) (Vance et al., 1987b). The predictions of CO_2 and MB-C_{SIR} from these two extracts were slightly improved when they were combined in the stepwise regression analysis (eq. 1–2). About 79.2% of the total variations in MB-C_{SIR} evolution were explained by:

$$\text{MB-}C_{SIR} = 1.19\ \text{SSC-F} - 0.380\ \text{SSC} - 35 \quad (1)$$

Similarly, about 66.0% of CO_2 evolution over six months was explained by eq. (2).

$$CO_2 = 0.277\ \text{SSC-F} - 0.070\ \text{SSC} - 26.0 \quad (2)$$

However, evacuation of chloroform should be done carefully as the smell did not disappear from some of the peat samples especially from the weakly humified ones.

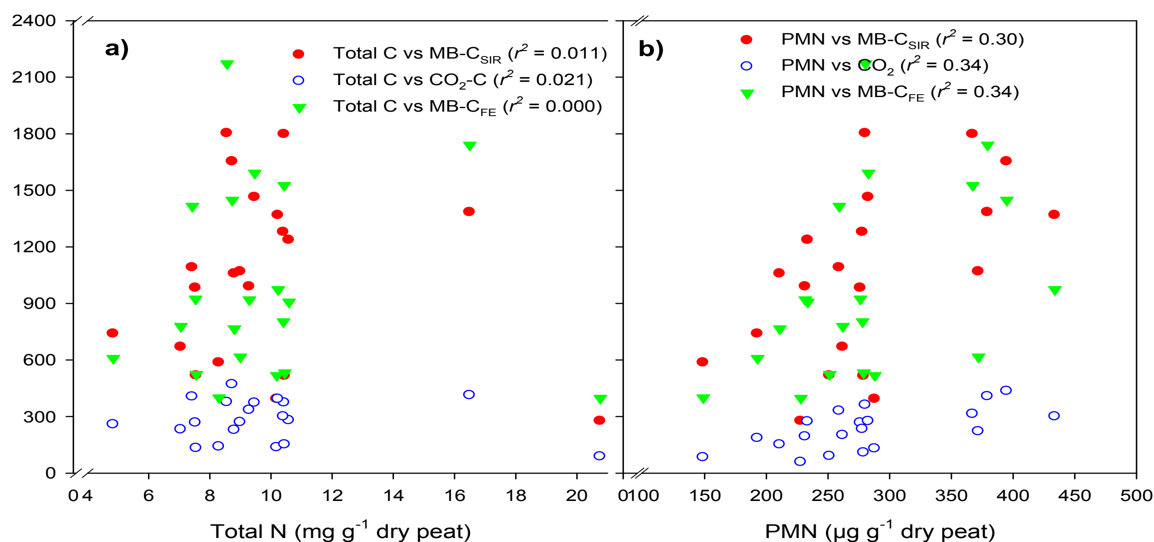

Fig. 3. Relationships of mean daily CO_2 evolution over six months (y-axis; μg g^{-1} dry peat d^{-1}) and microbial biomass-C (μg g^{-1} dry peat) estimated by the fumigation extraction (MB-C$_{FE}$) and substrate-induced respiration (MB-C$_{SIR}$) methods with a) total N and b) potentially mineralizable N (PMN).

Total N ranged from 0.48 to 2.01% (data not shown) and had no correlations ($r \leq 0.02$) with CO_2 evolution, MB-C$_{SIR}$ and MB-C$_{FE}$ (Fig. 3a). It appeared that potentially mineralizable-N had relatively better linear relationships ($r = 0.57$, $r = 0.65$ and $r = 0.44$) with the amount of and MB-C$_{SIR}$, microbial respiration and MB-C$_{FE}$, respectively (Fig. 3b).

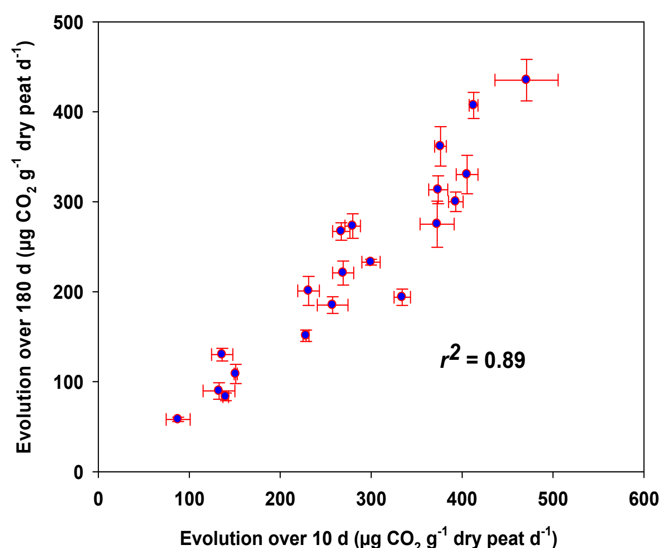

Fig. 4. Relationship between the mean daily evolved CO_2 over the first 10 d and the mean daily CO_2 evolution over 180 d. Each data point was the mean of three separate measurements, and the horizontal and vertical bars represented the standard deviations between the means for 10 and 180 d CO_2 measurements, respectively.

There was a strong linear relationship between the mean daily CO_2 evolution over 10 d (Basal respiration in chapter 2) and the mean daily CO_2 evolution over 180 d to suggest that microbial activity measured after 10 d is an indicator for OM degradation in peat samples over long-term (Fig. 4). However, mean evolved CO_2 over 10 d was relatively higher than the corresponding daily evolution over 6 months.

Based on results from the hierarchical cluster analysis (Fig. 5), the whole peat samples were grouped into three where the first cluster composed of peat samples with high amount of water- and salt-soluble OC, cluster *ii* contained peats with the least extractable OC, and cluster *iii* contained peats with dissolved OC that mostly occupied the middle ranges.

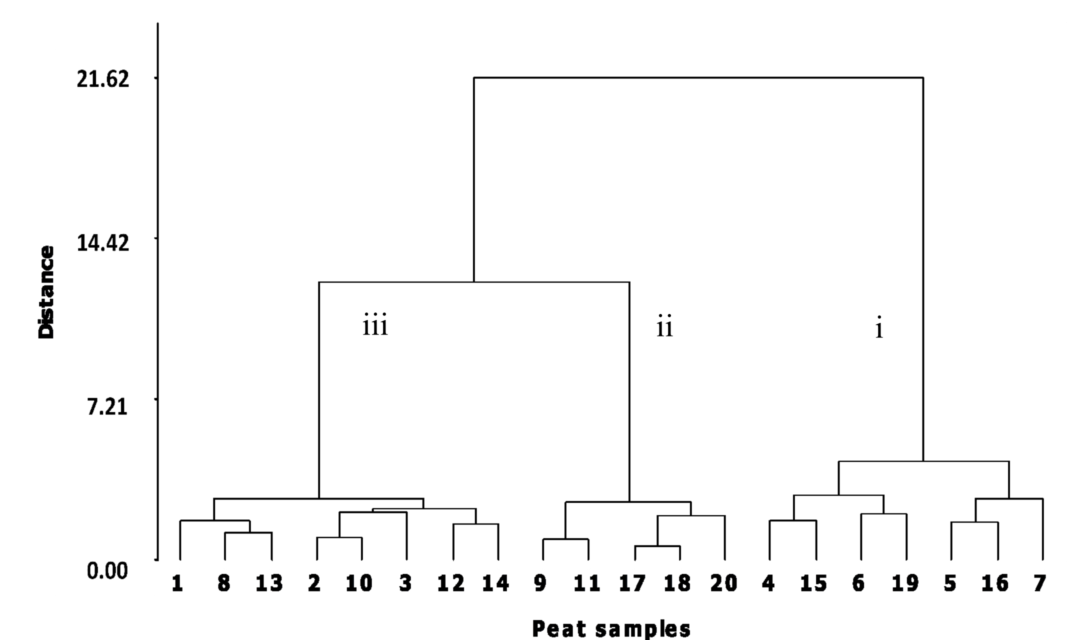

Fig. 5. Grouping of peat samples using data from the mean daily CO_2 evolution over six months, microbial biomass-C estimated by the fumigation extraction and substrate-induced respiration methods, total OC, cold-water extractable C, hot-water extractable C, salt-extractable OC, salt-extractable OC in hot-water bath and potentially mineralizable N. The hierarchical cluster analysis was established according to the Ward method based on the Euclidean square distance. See Table 1 for numbering of the peat samples.

3.5. Discussion

Since the natural organic matter (OM) in peats/soils mostly consists of organic materials in the following phases: a) partially degraded but still identifiable plant tissues; b)

microbial biomass; c) organic coatings of mineral phases; d) identifiable organic substances of low molecular weight; and e) the refractory part of OM (humic acid, fulvic acid, and humin) (Piccolo, 2001), the separation and quantification of different organic C pools is of particular importance for an understanding the most labile OC responsible for microbial decomposition, and C and N transformations in peat/soil samples. In this study, therefore, the total OM of twenty different peats were fractionated into total OC (TOC), cold-water extractable OC (CWC), hot-water extractable OC (HWC), salt-extractable OC (SSC), and salt-extractable OC in a hot-water bath (SSC-H) to investigate their relations with microbial activity (measured as evolved CO_2 over six months) and biomass-C of peat samples. Findings in Chapter 2 (it is also a case in this chapter) showed that microbial respiration had very weak correlations with humification degree (H), %N, pH, OM content, and the C/N ratio. Total OC also explained only 38% of the total variations in CO_2 production over six months, or 30 and 32% of the microbial biomass-C estimated by FE and SIR methods (Fig. 2), respectively, to indicate that much of the variations in the peat microbiological properties could not be explained by TOC measurement. Similar results were obtained for composts with different origins (bio- and green-composts) (Amha and Bohne, 2008).

McGill et al. (1986) have stated that water extractable OM is a pool of organic material with a high turnover rate; and it typically represented 0.3 to 1% of the total OM in agricultural soils (Jones et al., 2004). In this study, the mean CWC was minimal as compared to the agricultural soils and accounted for 0.024% of TOC (Table 2). The soluble organic C in cold-water extract of soil is often considered as easily decomposable organic compounds (Katz et al., 1985). Therefore, it was assumed that the CWC from peat is an easily degradable OC component and could explain much of the variations in microbial activity. However, it explained only 67% of the total variations in CO_2 evolution (Fig. 2c) indicating its limitation to predict the long-term microbial activity in peat samples. CWC also showed relatively weak correlations with MB-C estimated by SIR and FE methods as reported by van Ginkel et al. (1994) for mineral soils. This poor correlation can be explained by the presence of very little microbial cells in the cold-water extract. Shaking moist peat samples with 0.5 M K_2SO_4 (SSC) rendered a higher amount of OC than with cold-water (Table 2). However, such increase in dissolved OC was not translated into improved relationships with microbiological data (Fig. 2e).

Organic C extracts in both water and salt solution under hot-water bath showed greater correlations with the CO_2 evolution and biomass-C (Fig. 2 d & f). Fischer (1993) also found strong correlation between HWC contents in soils and CO_2 evolution, which would indicate that a proportion of the HWC must be easily available for microbial utilization. Boiling soil samples at 70 °C known to kill vegetative microbial cells (Sparling et al., 1998) to suggest that the procedure adopted here (boiling the samples in hot-water bath at 80 °C for 16 h) makes microbial biomass components extractable. However, the amounts of OC in SSC-H and HWC were 1.3 to 3.2-fold and 0.8 to 2.3-fold of the MB-C estimated by FE, respectively (Table 2), indicating that boiling peat samples in a hot-water bath would also extract appreciable amounts of the OC from non-biomass organic fractions.

The humification degree (H) has a major impact on the physical and chemical characteristics of peats (Puustjarvi and Robertson, 1975), and strongly related to their potential for horticulture. On the von Post humocity scale (Post, 1924), the H-values in the tested peats ranged from H3 to H7 (Table 1), and peat samples with lower degree of humification contained considerable amounts of fibrous material while the strongly humified peats showed amorphous structure with no or little fibrous matter. The H-values showed very poor correlations with CO_2 evolution ($r = -0.17$), MB-C_{FE} ($r = -0.28$) and MB-C_{SIR} ($r = -0.34$). However, these correlations were considerably biased by the eutrophic peats that showed the higher activity and biomass regardless of their respective higher H-values. The highest CO_2 evolution in the eutrophic peat might be explained by the presence of sufficient amount of easily decomposable organic compounds in the water or salt extracts (Table 2), which can be used as sources of electron donor by the heterotrophic microorganisms. The eutrophic peat, as summarized by Shotyk (1988) and Steinmann and Shotyk (1997), is generally rich in nutrient and the aboveground biomass in this peat-forming environment decomposed regularly and allows the accumulation of easily decomposable plant materials in the peat. Exclusion of these peat samples from the regression analysis could, therefore, improve the correlations to $r = -0.63$, $r = -0.54$ and $r = -0.57$, respectively.

Despite the smallest *r*-value, the inverse relationship between H and activity data also suggests that microbes in strongly humified peat types might have suffered, at least

partly, from the poor substrate quality. This assumption seems to be supported by the observed negative correlations between H and extractable OC (r = −0.50 to −0.63). The OM percentage also decreased with increasing H-level (r = −0.79) suggesting microbial communities in weakly humified peats may have more available metabolic carbon irrespective of their smaller TOC contents (Table 2) compared to the moderately or strongly humified peats. As noted by Buttler et al. (1994), Fisk et al. (2003) and Glatzel et al. (2003), a highly humified peat is mainly constituted sphagnan and other humic acids that do not have labile C and are poor energy sources. The general trend of OC compounds in peats shows that the carbohydrate content (i.e., a relatively easy to degrade compound) decreases as the humification process proceeds but humic substances increased. The above authors including Prasad and O'Shea (1997) also reported an inverse relationship between H and microbial respiration.

The hot-water extraction procedure has been suggested for determining the amounts of plant available N in soils (Keeney and Bremner, 1966). Unlike the total N (Fig. 3a), the potentially mineralizable N (PMN) had shown better correlations with microbiological data (Fig. 3b), and water and salt extractable OC (data not shown). The correlations were significant with HWC (r = 0.59) and SSC-H (r = 0.55) than with CWC (r = 0.46) and SSC (r = 0.41) to suggest that hot water bath extracted organic fractions composed of labile or mineralizable pool of organic N.

Overall, *Sphagnum* peat moss is generally perceived as one of the most stable organic potting media constitutes by growing media producers, as it is well decomposed over a long time. The resistance of *Sphagnum* remnants to decomposition is partly favoured by the presence of oxopolysaccharide (Painter, 1983) and specific secondary compounds such as polyphenols (Freeman et al., 2004) that suppress soil heterotrophs and extracellular enzyme activity. Peat samples included in this study, however, showed very distinct behaviours with regard to the size of dissolved OC, microbial activity and biomass-C. Based on results from the hierarchical cluster analysis (Fig. 5), they were clustered, but with very few exceptions, into three distinct groups: (i) peats with the lowest amounts of salt- and water-extractable OC producing <135 CO_2 g^{-1} dry peat d^{-1} with the corresponding biomass of <600 μg MB-C g^{-1} dry peat, (ii) peats with medium OC fractionates producing 135–270 CO_2 g^{-1} dry peat d^{-1} with MB-C of 600–1200 μg g^{-1} dry

peat, and (iii) peats with the highest amounts of salt- and water-extractable OC producing >270 CO_2 g^{-1} dry peat d^{-1} with MB-C of >1200 μg g^{-1} dry peat. Most peats from Finland produced the highest CO_2 whereas peats from Ireland and Germany (peat 17 and 18) produced the lowest CO_2 to suggest that the geographical location might have influence on the properties of peats apart from the widely used criteria to classify peats (i.e., the botanical composition, the humification degree and the peat-forming environments). Peats from the Baltic States but peat 4 and 20 occupied the middle ranges. Excessive decomposition of OM in the first group might, therefore, have unintended consequences when they are used in a long-term pot plant production by losing their physical structure (e.g. water and air capacity), mineralizing and/or immobilizing nutrients (e.g. nitrogen), and by changing the chemical behavior (e.g. pH, EC, cation exchange capacity) of the potting media (Prasad and O'Shea, 1997; Verhagen, 2009).

3.6. Conclusions

Microbiological data showed considerably higher correlations with all dissolved organic fractions to suggest that extraction of dissolved OC would provide greater insight into the labile OM responsible to the degradation of peat samples than the total OC (TOC) measurement. Specifically, OC extracted in hot-water bath (HWC and SSC-H) showed strong correlations with long-term evolved CO_2 (r = 0.94 and r = 0.92, respectively) that make both fractions can be used as indicators of microbial activity and biomass of peats. It should be noted, however, that the salt extracted OC showed less deviation within replications than water extracted OC to suggest a higher reproducibility of the SSC-H data. The eutrophic peats contained the highest mean dissolved OC followed by the oligotrophic, transitional and mesotrophic peat types. A strong linear relationship (r = 0.94) between the mean daily CO_2 evolution over 10 d and mean daily CO_2 evolution over 180 d indicating that microbial activity measured over 10 d is a surrogate to a long-term OM degradation in peat samples. The tested peats were broadly classified into three distinct groups based on results from the hierarchical cluster analysis.

4. Fungal and bacterial activity in peats using a selective inhibition technique

4.1. Abstract

Since the decomposition of organic carbon in peats is affected by the activity, biomass and composition of microorganisms, the relative contributions of fungi and bacteria to CO_2 evolution were assessed from 20 peat samples that vary greatly in their peat-forming environments (*eutrophic*, *oligotrophic*, *mesotrophic* and *transitional* types). Triplicate subsamples (each corresponding to 50 mL peat at 60% water filled pore space) were amended with different rates of streptomycin and cycloheximide (0–10 mg g^{-1} moist peat), and CO_2 was measured as a substrate-induced microbial respiration. Glucose and KNO_3 were added to each treatment to enhance microbial activity. The lowest concentrations of streptomycin and cycloheximide producing maximum inhibitions in peat samples were 3 and 4.5 mg g^{-1} moist peat, respectively. Evolution of CO_2 from the control treatments (0.8 g glucose-C and 0.4 g NO_3^-–N amended peat samples without the addition of antibiotics) ranged from 103 to 673 mg L^{-1} dry peat d^{-1}. Streptomycin addition inhibited microbial respiration by 5.5 to 53.5% while cycloheximide addition by 18.9 to 78.0%. Addition of both antibiotics as a mixture did not result in a complete inhibition of the substrate-induced respiration rate (it suppressed only by 39.9 to 86.7%). The calculated fungal-to-bacterial ratio and inhibitor additivity ratio (IAR) varied considerably between peat samples, and ranged from 0.46 to 9.96 and 0.76 to 1.48, respectively. The contributions of fungi were mostly higher in oligotrophic and eutrophic peats while bacterial dominance was higher in the transitional and mesotrophic peat samples. The relative proportions of fungal and bacterial activity were compressed to fit an IAR of 1.0, where mesotrophic and transitional peats mostly showed overlapping effects and the oligotrophic peats showed antagonistic effects.

Keywords: combined inhibition, cycloheximide, fungal-to-bacterial ratio, inhibitor additivity ratio, peat-forming environment, streptomycin

4.2. Introduction

Soil microorganisms are responsible for the majority of organic matter decomposition and mineralization in soils (Anderson and Domsch, 1975; Bailey et al., 2002; West, 1986), and preserve energy and nutrients in their biomass. They encompass various genera and species of fungi, bacteria, archaea and protozoa, and yet each of these groups of microorganisms can play unique roles in C and nutrient cycling. The abundance and composition of soil microorganisms can be influenced by factors related to soil and environmental conditions. Hu et al. (2001), for instance, reported fungal dominance in the nutrient limited environment over bacteria as the fungal population have higher C/N ratio than bacteria (McGill et al., 1986). The fungal-to-bacterial ratio tended to decline with the addition of N fertilizer (Bardgett and McAlister, 1999) and residues with low C/N ratio (Bossuyt et al., 2001). Although microorganism biomass is relatively low in peatlands, significant differences in both microbial diversity and microbial functional activity were reported among peatland habitats (Fisk et al., 2003). Golovchenko et al. (2007) also reported differences in microbial population among peatlands where the minerotrophic fen site dominated by bacteria and the ombrotrophic sites by fungi. Winsborough and Basiliko (2010), on the other hand, observed strong bacterial dominance in peats of three distinct peatlands (bog, rich fen and poor fen).

Sakamoto and Oba (1994) found that fungal-to-bacterial biomass ratio significantly affects the relationship between CO_2 evolution and the size of microbial biomass. Since bacteria and fungi exhibited differing resources utilization strategies (Poll et al., 2006), separation of the fungal and bacterial contributions to decomposition could thus help to understand how C and nutrient cycling are influenced in peat/soil. To this end, various methods are currently available to determine the fungal-to-bacterial ratio including the direct microscopy, selective inhibition (SI), extraction of cell membrane components (e.g. phospholipid fatty acid, ergosterol) as well as cell wall components (e.g. chitin and muramic acid) (Joergensen and Wichern, 2008). It should, however, be noted that each of these methods has its own advantages and limitations. Although the use of SI technique for peat samples is limited (Andersen et al., 2006), it has long been used to quantitatively partition glucose-induced bacterial and fungal respiration in agricultural

and forest soils (Alphei et al., 1995; Anderson and Domsch, 1975; Bailey et al., 2003). This method uses selective antibiotics to inhibit the activity of bacteria (mainly by streptomycin) and fungi (mainly by cycloheximide) under the following two basic assumptions: (i) the fungal-to-bacterial ratio in the antibiotic-sensitive biomass is the same as the ratio in the antibiotics-insensitive biomass, and (ii) bacteria and fungi respond similarly to the added substrate (i.e., glucose).

The success of SI technique is greatly relied on the right choice of antibiotics and their concentrations (Alphei et al., 1995). It has been reported that soils with higher organic matter contents and lower pH generally required higher concentrations of antibiotics (e.g. Ananyeva et al., 2006; Bailey et al., 2003; Lin and Brookes, 1999). Ananyeva et al. (2006), for instance, used higher rates of streptomycin (50–120 mg g^{-1} soil) and cycloheximide (50–80 mg g^{-1} soil) in the highly organic tundra, soddy-podzolic (coniferous forest, meadow, and arable), grey forest (larch forest), and chernozem (virgin and arable) soils to effectively inhibit bacterial and fungal respiration. Lin and Brookes (1999), however, achieved maximum inhibition in 13 arable, grassland and woodland soils with additions of relatively small amounts of streptomycin (4-8 mg g^{-1} soil) and cycloheximide (8-12 mg g^{-1} soil). Peats from different peat-forming environments (bog, poor fen and rich fen) also required different rates of antibiotics to inhibit substrate-induced microbial respiration (Winsborough and Basiliko, 2010), which emphasizes the need for antibiotics optimization. The objectives of this study were (i) to optimize the amount of glucose and antibiotics (streptomycin and cycloheximide) to be added in peat samples, (ii) to check the suitability of the selective inhibition technique for a wide range of peat samples, and (iii) to measure the fungal-to-bacterial ratio in peat samples.

4.3. Materials and Methods

4.3.1. Peat samples

Twenty peat samples were collected from different horticultural peat producing sites of Finland, Germany, Ireland and Sweden (Table 1). Peats from Baltic States (Estonia, Lithuania and Latvia) were also included, as they have recently gained considerable

importance as horticultural peat supplying countries. From each site, peat was collected in three separate bags and each bag was then considered as a replicate. Based on the peat-forming environments, these peats were classified as *eutrophic* (*n* = 2), *oligotrophic* (*n* = 9), *mesotrophic* (*n* = 3) and *transitional* (*n* = 6) peats (Stewart and Kantrud, 1971). Samples were sieved through a 5-mm sieve and thoroughly mixed before analysis. The details on the physical and chemical properties, and botanical composition of these peats were reported elsewhere (Amha et al., 2010). The water contents of the sieved samples were adjusted to 40% water filled pore space (WFPS) with distilled and deionized water. Prior to the selective inhibition experiment, peat samples were incubated under aerobic conditions for a week at 20 °C. This would allow equilibration of microbial activity following sample handling (sieving, storage and moistening).

Table 1
General descriptions of peat samples included in the study

Peat	Country	Bog name/area of extraction	H[x]	pH in water (1:5 w/v)	Dry bulk[y] density (g L^{-1})	Peat-forming Environment[z]
1	Latvia	Turkums	3	4.62	45	ombrotrophic bog
2	Lithuania	Gabolinus	3	4.42	44	ombrotrophic bog
3	Lithuania	-	3	4.60	46	fen-bog transition
4	Estonia	West Estonia	3	4.71	79	ombrotrophic bog
5	Finland	Lapua	3	4.28	58	ombrotrophic bog
6	Finland	Seinajoki	3	4.46	87	fen-bog transition
7	Finland	Närpio	3	4.60	54	ombrotrophic bog
8	Latvia	LaFlora	4	4.75	49	ombrotrophic bog
9	Ireland	Sharagh	4	4.91	61	mesotrophic fen
10	Lithuania	15b Laukas	4	4.77	74	fen-bog transition
11	Ireland	Brehony	4	4.62	54	ombrotrophic bog
12	Germany	Osnabrück	4	4.46	80	fen-bog transition
13	Estonia	Pütte-27	5	4.62	90	ombrotrophic bog
14	Sweden	Drakamyr	5	4.58	90	fen-bog transition
15	Germany	Vechta	5	4.80	109	mesotrophic fen
16	Finland	Kikilla	5	4.29	76	eutrophic fen
17	Germany	Vehmemoor	6	3.91	123	mesotrophic fen
18	Germany	Abesinien, P-2	7	4.74	107	ombrotrophic bog
19	Finland	Kikilla	7	4.97	170	eutrophic fen
20	Estonia	West Estonia	7	5.19	155	fen-bog transition

[x]according to von Post humification degree (von Post, 1924), where H1 designates a completely undecomposed peat and H10 as a completely decomposed peat.
[y]according to VDLUFA (2002).
[z]according to Stewart and Kantrud (1971).

4.3.2. Glucose and antibiotics optimization experiment

In this preliminary study, four moderately humified peats (peat 12, 13, 15 and 16; Table 1) were used to determine optimal glucose and antibiotics concentrations to be added in the inhibition experiment. According to Stewart and Kantrud (1971), these peat samples represented the *transitional*, *oligotrophic*, *mesotrophic* and *eutrophic* peat types, respectively. Optimum concentration of glucose was achieved by incubating 50 mL of moist peat samples with eight glucose concentrations (0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.2 C g L^{-1} dry peat). The amount of moist peat sample corresponding to 50 mL was determined according to VDLUFA (2002). Jars containing samples (each 1.5 L capacity) were then closed ~15 min after amendments, and incubated in the incubating chamber at 25 °C. The final water content was adjusted to 60% WFPS. The CO_2 concentrations in the headspace were measured (at the beginning and after 24 h) on a Perkin Elmer Autosystem XL gas chromatograph (GC) equipped with thermal conductivity detector (TCD). CO_2 production was calculated from a calibration line obtained with four standards, and expressed as mg CO_2 L^{-1} dry peat d^{-1}.

To determine optimum concentration of antibiotics, triplicate subsamples (10 g moist peat each) were weighed into 100 mL beakers and subsequently treated with six concentrations of streptomycin or cycloheximide solution (0, 1.5, 3.0, 4.5, 6.0 and 10 mg g^{-1} fresh weight). The antibiotics were thoroughly mixed with the samples using thin metal rod. All beakers were then sealed with parafilm (Parafilm®, Menasha, WI, USA) and incubated at room temperature for about 24 h. Since microbial respiration in peat is limited by the availability of both C and N (Martin and Holding, 1978), glucose and KNO_3 solutions were added to each treatment at rates of 0.8 g C and 0.4 g N L^{-1} dry peat, respectively. Thereafter, each beaker was kept inside a 1.5-L glass jar and incubated at 25 °C. The moisture contents in all treatments were adjusted to their respective 60% WFPS. Headspace gases were taken with syringes (0 and 24 h) and analyzed for CO_2.

4.3.3. Selective inhibition of fungal and bacterial activity

Once the amount of glucose-C, streptomycin and cycloheximide concentrations were determined (0.8 g, 3.0 mg and 4.5 mg g^{-1} moist peat, respectively), selective inhibition of

CO_2 evolution was performed according to Anderson and Domsch (1975) with some modifications. From each peat type, 10 g moist peat sample (n = 3) was amended with (A) glucose, (B) glucose + streptomycin, (C) glucose + cycloheximide, and (D) glucose + both antibiotics. All treatments also received KNO_3 at rate of 0.4 g NO_3^-–N L^{-1} dry peat. The final moisture content in each treatment was adjusted to 60% WFPS and incubated at 25 °C. Headspace gases were taken at initial and after 24 h and analyzed for CO_2.

4.3.4. Calculations and statistical analyses

The relative inhibitions of streptomycin, cycloheximide and their mixture to substrate-induced respiration were calculated from:

$$\text{Bacterial inhibition} = (A - B)/A * 100 \quad (1)$$

$$\text{Fungal inhibition} = (A - C)/A * 100 \quad (2)$$

$$\text{Combined inhibition} = (A - D)/A * 100 \quad (3)$$

where A, B, C and D are CO_2 evolution following addition of glucose + NO_3^-–N, glucose + NO_3^-–N + streptomycin, glucose + NO_3^-–N + cycloheximide and glucose + NO_3^-–N + streptomycin + cycloheximide, respectively.

Inhibitor additivity ratio (IAR) was also calculated as a measure of the inhibition of non-target organisms by one or both antibiotics (Bailey et al., 2002; Beare et al., 1990) from the equation

$$IAR = [(A - B) + (A - C)]/ (A - D) \quad (4)$$

An IAR of 1 would thus indicate there is no overlapping or antagonistic effect while IAR >1 illustrates the presence of overlapping antibiotic effect (i.e., substantial non-target inhibition by one or both antibiotics). Similarly, IAR becomes <1 when there is an antagonistic effect (i.e., the combined addition of antibiotics being more efficient than the separate addition). The fungal-to-bacterial ratio (F:B) was also calculated by:

$$F{:}B = (A - C)/(A - B) \quad (5)$$

Since the dry bulk density of the tested peat samples differed considerably (40–141 g L^{-1}; Amha et al., 2010), comparison of microbial activity was made on a unit volume basis. A single value in the tables or figures is the mean of three measurements. The coefficient of variations (CV, %) were calculated according to s/x, where s represents the deviation

between the samples means and *x* is overall mean value (R statistical software, version 2.10.1, 2009).

4.4. Results

4.4.1. Glucose and antibiotics optimization experiments

Four moderately humified peat samples (peat 12, 13, 15 and 16; Table 1) were used in the glucose optimization study. Despite differences in magnitude, microbial respiration in all peat samples was stimulated by the added glucose (Fig. 1). The rates of CO_2 evolution in peat 12, 15 and 16 showed steep increases with increasing glucose concentrations. However, this was happened only up to 0.2 g C L^{-1} in peat 13 whereby further glucose additions did not result in a significant CO_2 evolution.

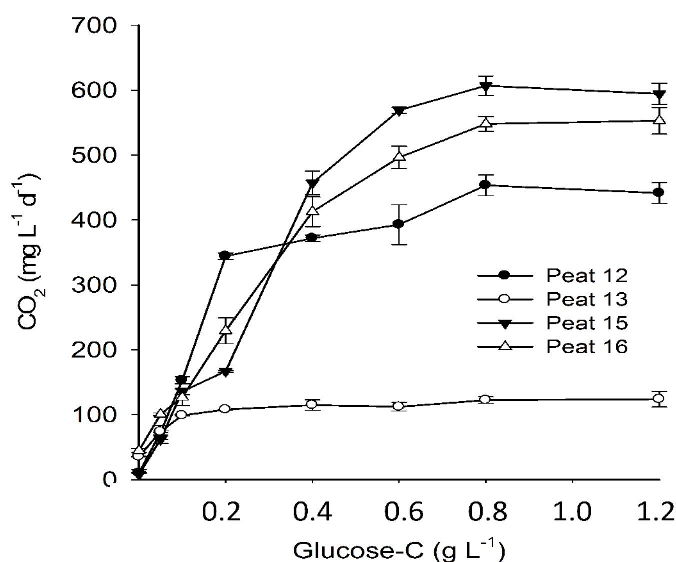

Fig. 1. CO_2-evolution from four moderately humified peat samples as influenced by the added glucose concentrations. The moisture content in each treatment was adjusted to 60% water filled pore space and incubated at 25 °C for 24 h. Each point was the mean of three measurements and the vertical bars indicate standard deviations. The description for peat samples is given in Table 1.

The concentration of antibiotics required for maximal inhibition varied between the tested peat samples (Fig. 2). Evolved CO_2 from peat 13 was slightly increased above the control (121 mg CO_2 L^{-1} dry peat) when streptomycin was added at rates of 1.5–6.0 mg g^{-1} moist peat (Fig 2a). The maximal inhibition in peat 16 was observed at lower

streptomycin concentration (1.5 mg g-1 moist peat) compared to peat 12 and 15 (4.5 and 3.0 mg g-1 moist peat, respectively). In contrast, the patterns of respiratory response to increasing concentrations of cycloheximide were comparable for all peat types (Fig. 2b). The activity of fungi was considerably suppressed by the addition of cycloheximide up to 4.5 mg g-1 moist peat although the inhibition in peat 16 was maximal at 6 mg g-1 moist peat.

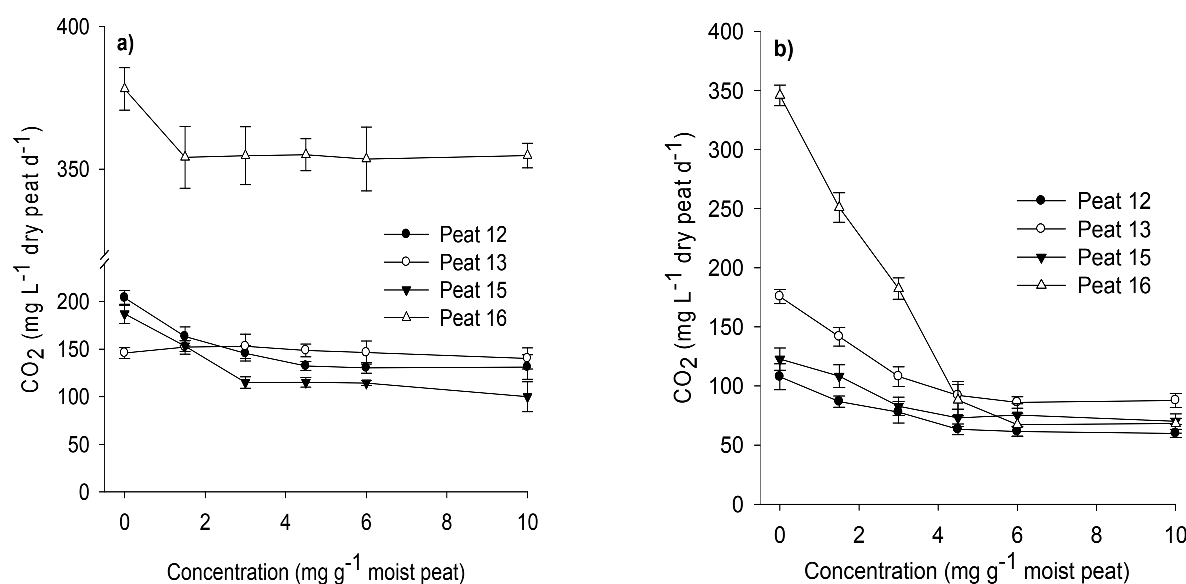

Fig. 2 Evolved CO_2 from four moderately humified peat samples after additions of streptomycin (a) and cycloheximide (b). Glucose and NO_3^-–N were added to each treatment at rates of 0.8 g C and 0.4 g N L^{-1} dry peat, respectively. The moisture contents in all treatments were 60% water filled pore space and incubated at 25 °C for 24 h. Each point was the mean of 3 measurements and the vertical bars indicate standard deviations. The description for peat samples is given in Table 1.

4.4.2. Selective inhibition experiment

The inhibition experiment was conducted using a wide range of peat samples (n = 20; Table 1). These peats varied greatly in their degree of humification, country of origin and the peat-forming environment. Evolution of CO_2 from the control treatments (i.e., moist peats that received 0.8 g glucose-C and 0.4 g NO_3^-–N L^{-1} but not antibiotics) ranged from 103 to 673 mg L^{-1} dry peat d^{-1} (Table 2). The rates of microbial respiration in six oligotrophic, two mesotrophic and four transitional peat samples were lower than the overall mean value of 227 mg CO_2 L^{-1} dry peat d^{-1}. In contrast, the highest CO_2 evolution (>300 mg L^{-1}) was measured from five peat samples: peat 4 (oligotrophic), peat 10 and 17 (transitional) and peat 16 and 19 (eutrophic).

Table 2

Percent of bacterial, fungal and combined inhibitions with respect to control, fungal-to-bacterial ratio (F:B), and inhibitor additivity ratio (IAR) in a wide range of peat samples subjected to antibiotics. All peat samples were received 0.8 g glucose-C and 0.4 g NO_3^-–N. The moisture content was adjusted to 60% water filled pore space and incubated at 25 °C

Peat Samples[a]	Control (mg CO_2 L^{-1} d^{-1})	% inhibition			F:B	IAR
		Streptomycin	Cycloheximide	Combined[b]		
1[O]	139 ± 8[c]	nd[d]	54.2 ± 1.9	67.6 ± 0.7	nd	0.77 ± 0.01
2[O]	136 ± 9	nd	46.6 ± 7.6	49.3 ± 7.0	nd	0.87 ± 0.02
3[T]	124 ± 16	40.4 ± 3.0	30.5 ± 2.0	53.6 ± 2.6	0.76 ± 0.01	1.32 ± 0.03
4[O]	318 ± 39	9.4 ± 1.0	73.3 ± 2.8	83.6 ± 2.8	7.88 ± 1.11	0.99 ± 0.01
5[O]	242 ± 16	12.3 ± 4.1	67.2 ± 10.2	82.0 ± 11.8	5.63 ± 1.07	0.97 ± 0.04
6[T]	264 ± 22	5.3 ± 0.1	38.3 ± 0.8	43.1 ± 0.3	7.21 ± 0.34	1.01 ± 0.02
7[O]	153 ± 10	8.1 ± 1.8	58.0 ± 5.0	67.3 ± 10.1	7.22 ± 0.94	0.99 ± 0.05
8[O]	180 ± 1	8.3 ± 2.6	36.2 ± 4.8	43.7 ± 4.0	4.48 ± 0.80	1.01 ± 0.08
9[M]	108 ± 5	49.1 ± 2.2	38.7 ± 3.2	59.5 ± 0.4	0.79 ± 0.10	1.48 ± 0.01
10[T]	372 ± 2	40.9 ± 3.0	18.9 ± 4.3	49.9 ± 5.4	0.46 ± 0.07	1.20 ± 0.02
11[O]	103 ± 3	19.9 ± 2.0	30.7 ± 5.2	39.9 ± 6.8	1.54 ± 0.10	1.27 ± 0.04
12[T]	156 ± 5	31.3 ± 4.2	37.5 ± 4.5	68.7 ± 8.5	1.20 ± 0.02	1.00 ± 0.00
13[O]	106 ± 38	nd	44.0 ± 1.6	52.9 ± 5.7	nd	0.76 ± 0.05
14[T]	212 ± 10	30.0 ± 0.2	27.0 ± 0.4	43.5 ± 3.8	0.90 ± 0.01	1.32 ± 0.10
15[M]	143 ± 17	44.1 ± 1.8	35.5 ± 4.1	61.2 ± 7.0	0.80 ± 0.06	1.30 ± 0.05
16[E]	340 ± 16	8.0 ± 0.3	78.0 ± 0.8	85.6 ± 3.3	9.76 ± 0.23	1.01 ± 0.03
17[M]	332 ± 15	37.5 ± 0.8	33.9 ± 3.2	55.5 ± 2.0	0.91 ± 0.11	1.31 ± 0.04
18[O]	242 ± 10	53.5 ± 2.1	38.0 ± 0.2	86.7 ± 3.9	0.71 ± 0.03	1.06 ± 0.03
19[E]	673 ± 58	7.3 ± 0.2	72.5 ± 1.8	78.2 ± 0.1	9.92 ± 0.04	1.02 ± 0.02
20[T]	196 ± 18	45.8 ± 2.0	28.2 ± 0.9	58.1 ± 3.1	0.62 ± 0.01	1.28 ± 0.12
Mean	**227**	**26.5***	**44.4**	**61.4**	**nd**	**1.10**
CV (%)	**59**	**65.6***	**38.6**	**24.9**	**nd**	**18.2**

[a]see Table 1 for the descriptions of the peat samples.
[b]combined addition of 3.0 mg streptomycin and 4.5 mg cycloheximide g^{-1} moist peat.
[c]mean ± SD (*n* = 3).
[d]not determined.
[E]eutrophic peat.
[M]mesotrophic peat.
[O]oligotrophic (ombrotrophic) peat.
[T]transitional peat.
*excluding peat 1, 2, and 13 as evolved CO_2 from streptomycin treated samples were higher than control samples.

Bacterial and fungal contributions to substrate-induced respiration with respect to the control were computed using *eq.* 1 and 2, respectively (Table 2). Bacterial respiration in three oligotrophic peats (peat 1, 2 and 13) was slightly increased (2.9, 4.4 and 3.8% of control, respectively) after the addition of 3.0 mg streptomycin g^{-1} moist peat. Bacterial inhibition in the remaining 17 peat samples ranged from 5.3 to 53.5%, with an overall mean value of 26.5%. Cycloheximide additions considerably depressed substrate-induced respiration in 12 peat samples while suppressions by streptomycin were higher

in eight peat samples. On average, 44.4% of substrate-induced respiration was suppressed by the addition of 4.5 mg cycloheximide g^{-1} moist peat (range = 18.9–78.0%; *n* = 20). Combined introduction of antibiotics into a wide range of peat samples reduced substrate-induced respiration by 39.9 to 86.7%, with overall mean value of 61.4% (Table 2).

The higher microbial activity after streptomycin addition resulted in negative fungal-to-bacterial (F:B) ratio in peat 1, 2 and 13 (Table 2). Excluding these peat samples, the F:B ratio in the mesotrophic and transitional peat samples (except peat 6 and 12) were between 0.46 and 0.91. In contrast, all oligotrophic peats but peat 18 had an F:B ratio of >1 indicating lower bacterial contributions to microbial activity. The F:B ratio in the two eutrophic peat samples were also exceptionally high (9.76 for peat 16 and 9.92 for peat 19). The presence of overlapping or antagonistic effect was compared on the basis of IAR (Table 2). With the additions of 3.0 mg streptomycin and 4.5 mg cycloheximide g^{-1} moist peat, IAR varied widely between 0.76 and 1.48. The relative proportions of fungal and bacterial activity were compressed to fit an IAR of 1.0 (Fig. 3), where transitional (except peat 12) and mesotrophic peats showed an overlapping effect and most oligotrophic peat samples showed an antagonistic effect. Based on the computed relative proportions, bacterial and fungal inhibitions ranged from 0 to 62.4% and from 17.4 to 90.7%, respectively.

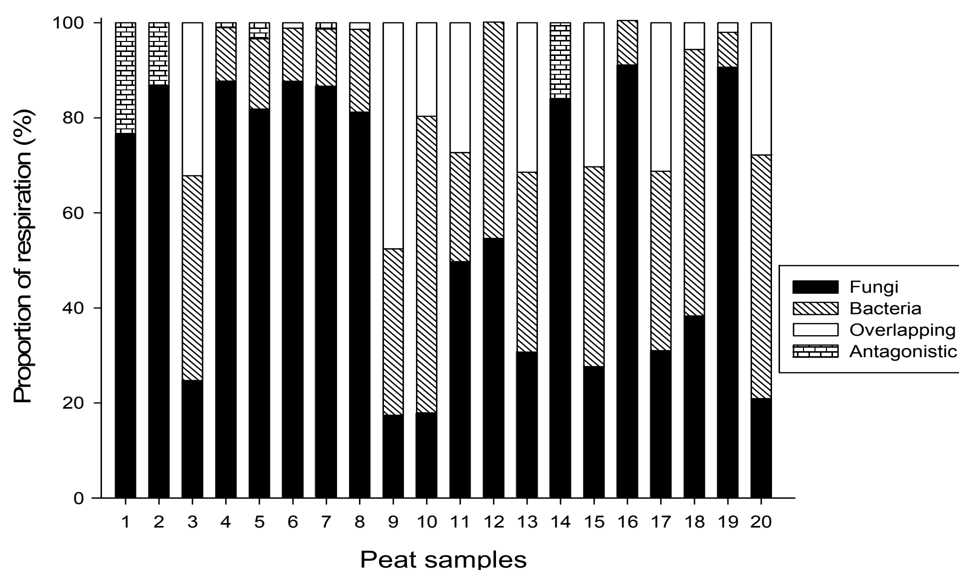

Fig. 3. The proportion of overlapping or antagonistic effect by the added antibiotics when compressed to an inhibitor additivity ratio of 1.

4.5. Discussion

The amount of glucose required to achieve maximum respiration rate could vary greatly between soils, mineral and organic types (Anderson and Domsch, 1975; Lin and Brookes, 1999; West, 1986). Results in Fig. 1 indicate that CO_2 production in all peat samples but peat 13 increased with increasing glucose concentrations and reached maximum at 0.8 g C L^{-1}. These evolved CO_2 from the peat samples are believed to be produced by glucose responsive microorganisms although glucose degrading extracellular enzymes (e.g. β-glucosidase) are naturally present in a range of peatlands (Fenner et al., 2005). It was, therefore, decided to use 0.8 g C L^{-1} (i.e., 2000 glucose µg mL^{-1} peat) in the selective inhibition experiment. Winsborough and Basiliko (2010), however, achieved the largest CO_2 evolutions with the addition of a much lower glucose concentration (667 µg glucose mL^{-1}) in bog, rich fen and poor fen peats. The chosen C rate in this study seems realistic as the concentration of dissolved organic C (DOC) in the rhizosphere soil reaches up to 750 µg g^{-1} (Bremer and Kuikmann, 1994). When computed in a volume basis (assuming the mean soil dry bulk density of 1.0 kg L^{-1}), the corresponding DOC is comparable to what was decided to be added here.

Optimizing the selective inhibition technique as a method for partitioning eukaryotic and prokaryotic activities may help to understand microbial control of respiration in peat soils and their respective peatlands (Winsborough and Basiliko, 2010). While substrate-induced respiration in peat 13 increased after streptomycin addition, it was decreased in other peats to indicate that the inhibitive effects of streptomycin on the bacterial population were variable between peat samples (Fig. 2a). Increasing streptomycin concentrations from 4.5 to 10 mg g^{-1} moist peat did not increase bacterial inhibition in all but in peat 15 suggesting inhibition of non-target organisms in peat 15. However, the responses of fungi to increasing concentrations of cycloheximide were similar and no significant inhibition observed beyond 4.5 mg (Fig. 2b). Therefore, addition of 3 mg streptomycin and 4.5 mg cycloheximide g^{-1} moist peat were considered to be the lowest concentrations producing maximum inhibitions in peat samples. The amount of cycloheximide added in this experiment was, however, considerably higher than Winsborough and Basiliko (2010) who achieved maximum fungal inhibition with an addition of 1.3 µg mL^{-1} bog, rich fen and poor fen peats. Since the activity of

microorganisms in their peat samples was dominated by bacteria (F:B of 0.34–0.68), they had rather added higher streptomycin concentrations (13–26 mg mL^{-1} peat). In general, soils with higher organic matter contents and lower pH required higher concentration of antibiotics (Alphei et al., 1995; Ananyeva et al., 2006; Bailey et al., 2003).

The use of selective inhibition technique in peats resulted in mixed responses (Table 2). Bacterial respiration in three of the nine bog peats were increased after bactericide additions elucidating that streptomycin may have been used as a source of energy by the non-target or resistant microorganisms. In literature, antibiotic utilization by non-target/resistant microorganisms (Badalucco et al., 1994) and adsorption/modification of antibiotic(s) by the soil components (Alphei et al., 1995; Lin and Brookes, 1999) are mentioned as plausible reasons for increased microbial activity after streptomycin addition. Suppression of bacterial activity in the remaining oligotrophic peat samples may, however, confirm greater variability within peats of the same origin/peat-forming environment. Dedysh et al. (2006) characterized the bacterial community in an acidic *Sphagnum* peat bog and includes *Acidobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Deltaproteobacteria*, *Chloroflexi*, *Verrucomicrobia*, and *Planctomycetes*.

The fungal-to-bacterial ratio in soils can be affected by physical disturbance (Hendrix et al., 1986), availability of nutrients (Bardgett and McAlister, 1999), soil pH, moisture gradient and temperature (Frey et al., 1999). It is generally calculated based on the antibiotic-susceptible soil fungal and bacterial community and ranged widely from 0.46 to 9.92 (Table 2). The F:B activity ratio of 1.0 indicates both fungal and bacterial population contributing equally to the microbiological activity of the soil sample (Bailey et al., 2002), which was not found in any of the tested peats. However, the contributions of fungi to substrate-induced respiration in most of the oligotrophic and eutrophic peats were greater than the corresponding bacterial contribution. According to Güsewell and Gessner (2009) and Högberg et al. (2007), the growth of fungi is favored by soils with acidic pH, low nutrient availability, recalcitrant organic matter and high C/N ratio compared to bacteria. The highest F:B ratio in the oligotrophic peats might, thus, be attributed to the prevailing conditions (e.g. oxygenated, nutrient poor) in the ombrotrophic environment (Shotyk, 1988; Steinmann and Shotyk, 1997). However, pH cannot be a reason here as almost all peat samples had acidic and nearly similar pH (Table 1). Bacteria dominate the activities of mesotrophic and transitional peat samples (Table 2), which is mostly in line with

Golovchenko et al. (2007) findings. They have observed bacterial dominance in minerotrophic peats (55–86%) and fungi dominance in the ombrotrophic sites (55–99%). Combined additions of streptomycin and cycloheximide did not result in a complete inhibition of microbial activity (39.9–85.6%; Table 2). This range is, however, higher than the mean values (40–70%) reported for most soils (Beare et al., 1990; West, 1986). The sources of evolved CO_2 from streptomycin and cycloheximide treated samples might be (i) survived fungal and bacterial populations and (ii) active constitutive enzymes in the samples (Heilmann et al., 1995). With regard to the later reason, *β*-glucosidase (Fenner et al., 2005) and phosphatase (Bonnett et al., 2006) are known to be present in wide range of peatlands. The evolved CO_2 might also come from non-target organisms, as microflora in peat composed of protozoa (e.g. testate amoeba), microalgae and micromotazoa (Payne et al., 2010).

The IAR is the sum of the respiration caused by the separate additions of the antibiotics divided by the respiration inhibition caused by their combined addition (Bailey et al., 2002; Beare et al., 1990). The computed range in Table 2 (IAR = 0.76 to 1.48) was higher than Winsborough and Basiliko (2010) who found an IAR of 1.06 to 1.17 for bog, poor and rich fens peats. However, IAR as high as 3.12 have been reported by Imberger and Chiu (2001). The relative proportions of fungal and bacterial activity were also compressed to fit an IAR of 1.0 (Fig. 3); and the results confirmed the occurrence of overlapping in mesotrophic and transitional peats (except peat 12) while antagonistic effects in most of the oligotrophic peat types.

4.6. Conclusions

The higher fungal contributions in the eutrophic and oligotrophic peats compared to the mesotrophic and transitional peat types suggesting microbial community structure in peats is greatly influenced by the peat-forming environments. Substrate-induced microbial respiration in mesotrophic and transitional peat samples were less suppressed by combined additions of streptomycin and cycloheximide (i.e., IAR > 1) than a separate addition of these antibiotics while the opposite observed in most of the oligotrophic peat samples (i.e., IAR < 1). These results emphasizing the importance of antibiotics optimization steps for each peat type. Overall, fungal and bacterial activity, IAR and the F:B ratio varied significantly between peat types, and such differences could have consequences on the C and nutrient cycling in these peat samples and their respective peatlands.

5. Evolution of CO_2 from peats amended with fertilizer, liming and binding materials

5.1. Abstract

Potting media producers and growers often add certain amount of additives (e.g. fertilizer, liming- and binding-materials) to peats to achieve an optimal rooting environment for plant growth. Four independent experiments were conducted to investigate the effects of pH (4.5, 5.5 and 6.5), nitrogen (KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3), carbon (glucose, cellulose and arginine), and glucose + NPK + micronutrients on microbial activity using a wide range of peat samples (n = 20). Evolved CO_2 from all peat types tended to increase when their respective pH increased from 4.5 to 5.5 units. However, microorganisms in the weakly humified peats responded strongly for pH increases from 5.5 to 6.5, indicating these peats could not maintain their stability at a higher pH compared to the moderately and strongly humified peats. Although peats were different in their responses, addition of glucose into all peat samples stimulated CO_2 production by 78 to 514% of control over 28 days of incubation period. These increases, however, did not confirm the presence of positive priming effect (i.e., accelerated intrinsic organic matter decomposition). Microbial activity in arginine treated peats was higher than control (36 to 294%), where it reached maxima between day 3 and 10. Cumulative CO_2 emissions in the N treated peat samples were lower than in the control (by -7.3 to -75.8%) regardless of the type of N-forms used, suggesting that decreases in respiration rates were mainly a direct result of the increase N availability than indirect effects caused by the form of N added. The responses of microorganisms for the added cellulose were positive although the observed increases (15 to 146% of control) were considerably lower than glucose and arginine treated samples indicating that the inclusion of cellulosic containing potting mixes (e.g. wood bark, fibers, and saw dust) into the tested peat samples may have little effect on the bio-stability of the final mixes. After 28 days of incubation period, addition of easily decomposable C source (glucose) together with a set of macro- and micro-nutrients had produced higher amounts of CO_2 in five peat samples compared to the amounts of CO_2-C in added glucose, indicating a positive priming effect.

Key words: arginine, cellulose, CO_2 evolution, glucose, N-fertilizer, pH

5.2. Introduction

The production of quality crops in greenhouse and nursery can be affected by a number of factors including the right choice of potting media. Because of its optimum water holding capacity (Wilson et al., 2003), adequate porosity (Bohne and Wrede, 2005), and unique combination of chemical properties (Reinikainen, 1997), peat is one of the most preferred potting media by nursery crop growers. As a result, large volumes of peats are being harvested from different peatlands by either milling, sod cutting, bucket excavation, or other means. The International Peat Society (IPS) survey report, for instance, indicated that the annual total production of peat was ≈90 million cubic meters (EPAGMA, 2005), of which 40% was designated for horticultural uses. Apart from the presence of adequate air and water contents, peat-based potting media should also maintain stability over the growing season. This is because, uncontrolled decomposition of peats in the container is known to have a direct influence on plant growth (Prasad and O'Shea, 1997; Prasad and Maher, 2008) by decreasing the total porosity, altering the chemical properties (pH, electrical conductivity), and releasing new organic compounds that could have phytotoxic or stimulating effects.

The stability of peat-based potting media during plant growth can be influenced by the use of *additives*. According to Schmilewski (2003), additives in the potting medium can be liming materials, fertilizers, binding materials (starch, cellulose, polyacrylates), buffering materials (clay, zeolite, aluminum oxide), wetting agents, hydrogels, pesticides, biocontrol agents, biostimulants, dyes, and others. Each of these additives plays significant roles in determining the physical, chemical, and biological properties of the final potting mixes. For instance, the inclusion of liming material to peat-based media could increase the availability of nutrients for plant and microorganisms. However, to what extent the changes in pH are likely to influence the stability of peats of contrasting properties is not yet well known. In this study, therefore, changing pH from acidic to slightly neutral pH assumed to increase the activity of microorganisms in peat-based potting media as pH influences many physical, chemical and biological properties and processes in soils (Brady and Weil, 1999). It was also assumed that microorganisms in

the weakly humified peats (≤H3) responded differently than the moderately (H4–H6) and the strongly (≥ H7) humified ones.

N fertilizers are also commonly added into the peat-based media. Since the assimilation and mineralization of C are generally influenced by the availability of inorganic nutrients, in this study, additions of different N-fertilizer sources assumed to accelerate microbial activity in wide ranges of peat samples. Apart from the low nutrient contents (Clymo, 1983), a large pool of organic matter in peat is not in a readily available form for microbes (Bridgham and Richardson, 1992) to suggest that microbial activity in peat is C-limited. It is, therefore, assumed that addition of binding materials like cellulose or other C-sources (e.g. glucose and arginine) may lead to an increase in organic matter decomposition of peat samples. Unlike cellulose and glucose, addition of arginine could also render N. The aim of this particular study was to investigate the effects of additives (fertilizer, liming and binding materials) on the activity of microorganisms using peats of different origins.

5.3. Materials and Methods

5.3.1. Peat sampling and handling

The responses of twenty peat samples to different pH, and C and N additions were tested under laboratory conditions. These peats were collected from different areas of Finland, Germany, Ireland and Sweden. Moreover, peats from Baltic States (Estonia, Lithuania and Latvia) were included as they have recently gained considerable importance as peat supplying countries. From each site, a bulk peat sample was collected in three different bags and inert materials (wood, stone) were removed. They were then sieved separately through a 5-mm screen. Peat materials, which had a size of >5 mm, were broken down and incorporated into the respective bulk sample. Sieved bulk samples were homogenized by hand-mixing. Because of a large number of peat types included in this study, the bulk samples were stored in plastic bags (at 4 °C) for several weeks. Each bag served as a replicate.

5.3.2. Samples preparation for incubation

The bulk sample in each bag was homogenized before to be used in subsequent incubation experimentations. Some subsamples were taken from each peat type and their respective moisture contents were adjusted to 40% water-filled pore space (WFPS; *eq.* 1) by adding distilled and deionized water (if necessary).

$$WFPS = \frac{W_m * D_{BD}}{\rho H_2O * P_S} * 100 \quad (1)$$

where, $WFPS$, W_m, D_{BD}, ρH_2O and P_S represent water-filled pore space (%), gravimetric water content (Mg/Mg), peat dry bulk density (Mg m^{-3}), density of water (Mg m^{-3}) and total pore space, respectively. The homogenized subsamples were then monitored for their microbial activity (measured as evolved CO_2) in subsequent incubation experiments.

5.3.3. Effect of C-sources on microbial activity (*exp. I*)

Triplicate subsamples (corresponding to 100 mL each) were taken from each conditioned peat type and subjected to the following C-amendments: (1) no C input; (2) glucose, (3) cellulose, and (4) arginine. Glucose was included as it is one of the most widely used C-sources in microbial degradation studies (Degens and Sparling, 1996) while cellulose was included to have a better understanding on the response of microorganisms to non-decomposed plant components in the potting media especially when fibrous materials are incorporated to improve aeration. Arginine was used to to evaluate the response of microorganisms to C- and N-containing organic compounds. All C-sources were added in the powder form at the rate of 0.4 g C L^{-1}. The C-amended subsample was mixed thoroughly in a plastic bag for a couple of minutes to achieve a uniform C distribution. Each treatment was packed in 100 mL pot and the respective moisture content adjusted to 60% WFPS. The pots were placed inside 1.5-L glass jars, sealed (with caps, sellotape and clips) and incubated for 28 d at 25 °C. Headspace gas was sampled at the beginning and after 1, 2, 3, 4, 7, 10, 14, 21, and 28 d of incubation period. Once the headspace gas is removed (10 mL/sampling), equivalent amount of ambient air was injected back into the closed jar to return the jar to its original pressure.

Computerized data loggers controlled the temperature of the incubation chamber (Rumed®, Rubarth Apparate GmbH, Germany) continuously.

5.3.4. Effect of N-sources on microbial activity (*exp. II*)

Moist equilibrated peat samples (n = 3; each equivalent to 100 mL) were treated with one of the three N-sources (NH_4NO_3, $(NH_4)_2SO_4$ and KNO_3) at rate of 0.4 g N L^{-1} dry peat. These N-sources are commonly used in nurseries and greenhouses for plant production. Fertilizers were ground to a fine powder and mixed thoroughly with the peat samples. Based on the pre-determined dry bulk densities, peats in all treatments were packed separately into 100 mL pots and the final water contents adjusted to 60% WFPS. Each pot was kept inside a 1.5-L glass jar, sealed with clips, and incubated at 25 °C. Headspace samplings (size and sampling intervals) were similar to *exp. I*.

5.3.5. Effect of pH on microbial activity (*exp. III*)

The effect of pH on microbial respiration was studied at three different pH levels (4.5, 5.5 and 6.5). Equilibrated peat samples (each equivalent to 50 mL peat) were weighed into the beakers and their respective moisture contents were adjusted to 150% WFPS. Then, 1 M H_2SO_4 or 1 M NaOH necessary to change the pH to target values was added while the slurries were vigorously stirred with magnetic stirrer. Each beaker was then kept inside a 1.5-L glass jar, sealed and incubated for 24 h. The headspace gas was sampled at the beginning, and after 1, 3, 6, 9, and 24 h of incubation period using airtight plastic syringe. Once the headspace gas is removed (10 mL/sampling), equivalent amount of ambient air was injected back into the closed jar to return the jar to its original pressure. The incubation temperature was 25 °C.

5.3.6. Peat amended with glucose and nutrients (*exp. IV*)

Triplicate subsamples (each corresponding to 100 mL) were then taken and amended with 10 mL of a macronutrient solution containing NH_4Cl (4.31 g L^{-1}), $CaCl_2$ - $6H_2O$ (5.39 g L^{-1}), $MgSO_4$ - $7H_2O$ (4.31 g L^{-1}), and 0.2 mL of a micronutrient solution containing $MnCl_2$ - $4H_2O$ (500 mg L^{-1}), $FeCl_3$ - $4H_2O$ (2000 mg L^{-1}), $CuCl_2$ - $2H_2O$ (30 mg L^{-1}), $ZnCl_2$

(50 mg L^{-1}), H_3BO_3 (50 mg L^{-1}), EDTA (1000 mg L^{-1}) and 36% HCl (1 mL L^{-1}) (Kargi and Serkan, 2006 as cited by Grigatti et al., 2007). K_2HPO_4 and KH_2PO_4 were also added to the macronutrient solution at rate of 2.5 g L^{-1} each. However, additional micronutrients described in Grigatti et al. (2007) were not used. The pH of the solution was adjusted to 5.7 using H_2SO_4 and NaOH. To ensure a better microbial activity, glucose was added at rate of 0.4 g C L^{-1} dry peat and all samples incubated at 25 °C for 28 d. Head space samplings (size and sampling intervals) were similar to *exp. I*.

5.3.7. Laboratory analysis

The headspace gases were taken using air-tight syringes and the respective CO_2 concentrations were determined by gas chromatograph (Perkin Elmer Autosystem XL, Country) equipped with thermal conductivity detector (TCD). The oven was heated at 35 °C and helium was served as a carrier gas. Peak area for CO_2 was determined by electronic integration and conversion of this area to µL CO_2 was made by measuring standard gases with the known CO_2 concentrations (200, 5000, 10000 and 20000 ppm). The GC showed a deviation of less than 5% for multiple injections. The volume of headspace was determined gravimetrically by filling the jar-containing sample with water to capacity (1.5 L).

5.3.8. Statistical analyses

The rate of evolved CO_2 (µg CO_2 g^{-1} dry peat d^{-1}) was calculated from the cumulative CO_2 concentration, headspace volume, incubated dry sample, and time of incubation period. CO_2 evolution in the control sample was subtracted from the corresponding C and/or N treated samples to estimate the effect of the added C- and N-sources for evolved CO_2. Since C- and N-isotopes were not used in this experiments, the effects of C and N additions were estimated by assuming the rate of intrinsic organic matter degradation in amended sample is similar to the corresponding unamended sample. ANOVA was performed and treatment means were separated by the Tukey comparison test ($P \leq 0.05$) using the SAS software (SAS instit., Inc. 9.1 for Windows, Cary, NC).

5.4. Results and Discussion

5.4.1. Control peat samples

The mean microbial respiration in the control (unamended) peat samples ranged from 77 to 440 µg CO_2 g^{-1} d^{-1} (Table 1). With some exceptions, peats from Finland mostly produced the highest CO_2 evolution followed by peats from the Baltic Nations. However, the two German (peat 17 and 18), one Estonian (peat 20), one Swedish (peat 14), and two Irish peats (peat 9 and 11) produced the lowest mean daily CO_2 evolution. The lowest CO_2 concentrations in these peat samples can partly be explained by the presence of small amounts of water- and salt-extractable OC (Chapter 3) that would have been used by microorganisms as sources of electron donor. Poor OC quality (recalcitrant type; Buttler et al., 1994) can also be another reason as these peats were moderately and strongly humified types on the von Post scale (von Post, 1924).

5.4.2. Microbial activity in C amended samples

Glucose is the most commonly used compound in biodegradation studies (Lin and Brookes, 1999); and its addition to soil mostly favors the development of fast growing microorganisms that are less effective in utilizing more resistant C pools compared to indigenous biomass (Wu et al., 1993). CO_2 production in all peat types was significantly increased after glucose addition (Table 1), and ranged from 475 (peat 20) to 1094 µg CO_2 g^{-1} dry peat d^{-1} (peat 4) with an overall mean value of 797 µg CO_2 g^{-1} dry peat d^{-1}. CO_2 evolution in the control samples were subtracted from the corresponding glucose treated samples to estimate the effect of the added C for evolved CO_2. The maximum CO_2 increases were observed in peat 9, 11, 14, 17, 18, and 20 to confirm the previous suggestions that the quantities of easily degradable OC in these peat samples are relatively low and/or the qualities are poor. These results indicating that mixing easily degradable C containing constitutes (e.g., immature compost) or other additives to peats significantly increase microbial activity, and eventually affect the structural stability of the final potting-mix.

Assuming a complete aerobic respiration inside the incubation jar, the amount of glucose mineralized over 28 d varied between 37.6 and 94.3% (Fig. 1); and did not confirm the presence of increased degradation of native OC in all peat types. This means that the loss of C through CO_2 evolution was less than that of C added to the peat samples. However, the validity of this assumption might be in question as we measured appreciably amount of denitrified (N_2O+N_2)–N at 60% WFPS (Amha and Bohne, 2011), indicating the presence of anaerobic microsites at the current incubation conditions. This assumption also ignored the incorporation of glucose-C into microbial biomass, although the direct use of glucose for microbial growth is reported to be between 40 to 60% (Paul and Clark, 1996). However, Hamer and Marschner (2002) measured accelerate mineralization of native organic matter (i.e., a positive priming effect) with addition of glucose at rate of 400 μg C g^{-1} peat. When expressed per gram basis, the amounts of glucose added here were quite higher than Hamer and Marschner (2002) work and ranged from 2.8 to 10.0 mg g^{-1} dry peat. The maximum additions were in the weakly humified peat samples as these peats had lower dry bulk density (Amha et al., 2010). The added glucose amounts were generally lower than the amount known to inhibit microbial respiration in peats (i.e., 60 mg glucose g^{-1} by West and Sparling (1986)) and in arable, grassland and woodland soils (i.e., above 71 mg glucose g^{-1} soil by Lin and Brookes (1999)). The percent of utilized glucose showed a positive correlation with the degree of humification to confirm that microbial activity in strongly humified peats was limited by the availability of easily degradable C sources.

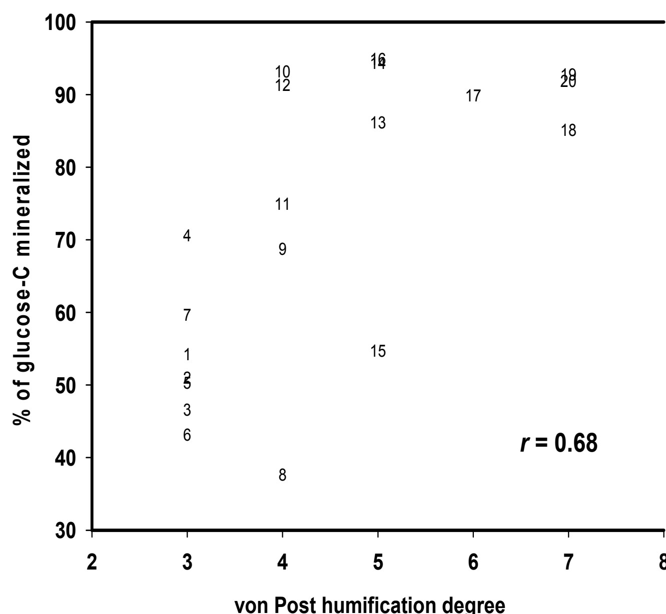

Fig. 1. Relationship between the percent of added glucose-C mineralized after 28 days and the von Post humification degree. The percent of glucose-C mineralized was calculated by assuming there is no difference in the rates of organic matter degradation between glucose –C treated and untreated samples. The numbers in the graph show peat samples as described in Table 1.

Table 1

Daily CO_2 evolution (CO_2 g^{-1} dry peat d^{-1}) from peats amended with different C-sources. The moisture contents in the tested peat samples were adjusted to 60% of the respective water filled pore space and incubated at 25 °C for 28 days

Peat	Country	H[a]	pH in water (1:5 w/v)	µg CO_2 g^{-1} dry peat d^{-1}				Increase (% of control)[b]		
				Control	Glucose	Cellulose	Arginine	Glucose	Cellulose	Arginine
1[O]	Latvia	3	4.62	234 ± 6	927 ± 95a	542 ± 23c	629 ± 17b	296	132	169
2[O]	Lithuania	3	4.42	246 ± 12	914 ± 160a	544 ± 39c	668 ± 2b	271	121	171
3[T]	Lithuania	3	4.60	197± 9	792 ± 85a	443 ± 34c	600 ± 17b	302	125	205
4[O]	Estonia	3	4.71	290 ± 1	1094 ± 35a	636 ± 24c	814 ± 67b	277	119	180
5[O]	Finland	3	4.28	314 ± 17	830 ± 21a	587 ± 25c	426 ± 5c	165	87	36
6[T]	Finland	3	4.46	379 ± 10	850 ± 100a	565 ± 18b	887 ± 83a	124	49	134
7[O]	Finland	3	4.60	343 ± 5	980 ± 39a	593 ± 21b	669 ± 95b	186	73	95
8[O]	Latvia	4	4.75	252 ± 7	700 ± 47a	441 ± 17c	738 ± 11a	178	75	193
9[M]	Ireland	4	4.91	110 ± 11	665 ± 14a	201 ± 20b	434 ± 23b	504	82	294
10[T]	Lithuania	4	4.77	283 ± 24	1012 ± 64a	566 ± 13c	485 ± 0c	257	100	71
11[O]	Ireland	4	4.62	105 ± 4	605 ± 17a	183 ± 17c	357 ± 17b	478	75	241
12[T]	Germany	4	4.46	239 ± 8	953 ± 8a	411 ± 31c	682 ± 88b	299	72	185
13[O]	Estonia	5	4.62	241 ± 15	702 ± 9a	592 ± 35ab	641 ± 34ab	191	146	166
14[T]	Sweden	5	4.58	176 ± 11	786 ± 24a	387 ± 49b	447 ± 40b	347	120	155
15[M]	Germany	5	4.80	358 ± 6	773 ± 34a	410 ± 18c	555 ± 8b	116	15	55
16[E]	Finland	5	4.29	434 ± 4	848 ± 98a	536 ± 24c	663 ± 27b	95	23	53
17[M]	Germany	6	3.91	133 ± 6	614 ± 86a	241 ± 11b	268 ± 10b	360	81	101
18[O]	Germany	7	4.74	122 ± 10	629 ± 33a	212 ± 17c	167 ± 22b	415	74	37
19[E]	Finland	7	4.97	440 ± 5	785 ± 55a	512 ± 5b	753 ± 46b	78	16	71
20[T]	Estonia	7	5.19	77 ± 3	475 ± 67a	154 ± 7b	180 ± 19b	514	99	132

[a]degree of humification according to von Post (1924).
[b]calculated as (glucose amended sample – control) x 100/control.
[O]oligotrophic (ombrotrophic) peat.
[E]eutrophic peat.
[M]mesotrophic peat.
[T]transitional peat.

All cellulose-treated peat samples had produced additional CO_2 over 28 d of incubation period (15 to 146% of control; Table 1). However, the relative increases were considerably low at the beginning of the incubation period (up to day 4) suggesting that microorganisms were not triggered by the added cellulose-C (see general discussion). The relative CO_2 evolution in cellulose-amended peat sample tended to increase in the following days, which was complemented by a decrease in evolved CO_2 from the respective control sample. CO_2 evolution in cellulose treated samples was, however, by far lower than the corresponding glucose received samples, suggesting both C-sources might have triggered different microbial population. Killham (1994) stated that the decomposition of cellulose is a relatively specialized depolymerization exercise (involving a restricted number of saprophytes) followed by hydrolysis to the simple glucose molecule. This makes the half-life and turnover time of cellulose in soil in the order of days or weeks compared to glucose metabolism, which is in the order of hours or a day. High molecular weight polymers such as cellulose do not induce the trigger response (De Nobili et al., 2001), presumably because their solubility is too low. The inclusions of relatively less degradable potting media constitutes with higher cellulosic contents (e.g. wood bark, fiber, sawdust) into peats might, therefore, not initiate massive increase in microbial activity over short time.

Microorganisms catabolise arginine via one or more of four major pathways: (1) the arginine-urease or arginase-urea amidolyase pathway, (2) the arginine transmidinase pathway, (3) the arginine deiminase pathway, (4) the arginine decarboxylase pathway. Except in the arginine transmidinase pathway, ammonium is an end-product (Abdelal, 1979 as quoted by Lin and Brookes, 1999). The rates of evolved CO_2 from arginine amended peat samples (167 to 887 µg CO_2 g^{-1} dry peat d^{-1}) were higher than control and cellulose treatments but considerably lower than the corresponding glucose treated peat samples (Table 1). Since arginine contains significant quantities of both C (41%) and N (32%), less response of microbes to arginine (compared to glucose) might suggest that these two organic C sources activated different microbial population. Maximum respiration rates from all peat samples were measured between day 3 and 10 (see general discussion). However, arginine desamination could result in NH_4^+ liberation even over a short incubation period (2 h) (Chapter 2).

Table 2

CO_2 evolution from peat samples amended with three inorganic N sources (NKO_3, $(NH_4)_2SO_4$, NH_4NO_3), and incubated at 25 °C for 28 d. The moisture contents of the incubated samples were adjusted to 60% of the respective water filled pore space

Peat sample	μg CO_2 g^{-1} dry peat d^{-1}			*P*-level	Evolved CO_2 (% of control)		
	KNO_3	$(NH4)_2SO_4$	NH_4NO_3		KNO_3	$(NH4)_2SO_4$	NH_4NO_3
1[O]	170 ± 18 [a]	188 ±24	170 ± 20	ns[b]	−27.4	−19.7	−27.4
2[O]	171 ±19	155 ±10	193 ± 29	ns	−30.5	−37.0	−21.6
3[T]	137 ±32	169 ±22	155 ± 52	ns	−30.4	−14.1	−21.2
4[O]	242 ±41	224 ±26	218 ± 8	ns	−16.7	−22.9	−24.9
5[O]	232 ±17	187 ±17	209 ± 10	ns	−26.0	−40.4	−33.4
6[T]	243 ±15	294 ±10	327 ± 22	ns	−35.8	−22.4	−13.7
7[O]	147 ±10	143 ±17	262 ± 11	ns	−57.1	−58.3	−23.6
8[O]	104 ±17	188 ±13	179 ± 13	ns	−58.7	−25.4	−29.0
9[M]	98 ±15	90 ±21	79 ± 20	ns	−11.0	−18.3	−28.3
10[T]	176 ±24	148 ±14	239 ± 36	ns	−37.9	−47.8	−15.7
11[O]	95 ±13	97 ±19	71 ± 4	ns	−9.2	−7.3	−32.1
12[T]	77 ±6	79 ±15	90 ± 13	ns	−67.8	−67.0	−62.4
13[O]	103 ±10	99 ±11	148 ± 4	ns	−57.3	−59.0	−38.6
14[T]	65 ±38	100 ±16	117 ± 23	ns	−63.0	−43.1	−33.4
15[M]	96 ±18	88 ±23	91 ± 7	ns	−73.2	−75.4	−74.6
16[E]	105 ±16	123 ±18	170 ± 12	ns	−75.8	−71.7	−60.9
17[M]	95 ±22	121 ±38	108 ± 8	ns	−28.7	−9.2	−19.0
18[O]	108 ±10	101 ±16	76 ± 5	ns	−11.5	−17.2	−37.7
19[E]	241 ±59	166 ±27	205 ± 7	ns	−45.2	−62.3	−53.4
20[T]	48 ±18	53 ±12	54 ± 7	ns	−37.9	−31.5	−30.2

[a]mean ± standard deviation (n = 3).
[b]*P*-level was higher than 0.05.
[O]oligotrophic (ombrotrophic) peat.
[E]eutrophic peat.
[M]mesotrophic peat.
[T]transitional peat.

5.4.3. Microbial activity in N amended samples

Large quantities of N in the form of nitrate (NO_3^-–N), ammonium (NH_4^+–N), and a combination of the two are used in horticulture to increase crop productivity. The rates of CO_2 evolution from KNO_3, $(NH4)_2SO_4$ and NH_4NO_3 treated samples ranged from 48 to 243 μg CO_2 g^{-1} dry peat d^{-1}, from 53 to 294 μg CO_2 g^{-1} dry peat d^{-1}, and from 54 to 327 μg CO_2 g^{-1} dry peat d^{-1}, respectively (Table 2). Although the initial C/N ratio were considerably reduced from 24–101 to 21–40 following N additions, the added N significantly suppressed the activity of microbes (7.3 to 75.8% of control) with no statistical difference ($P > 0.05$) between N forms used. Moreover, the computed *r*- values between measured CO_2 from the N-amended peat samples and their respective humification degree (r = −0.12 to −0.38) did not show the presence of any detectable

pattern about the effects of N on different peat samples. In their work, Ramirez et al. (2010) have recently reported a net reduction of microbial respiration (up to 60%) after treating three contrasting soils with six different inorganic N-sources (NH_4Cl, $(NH_2)_2CO$, $Ca(NO_3)_2$, and those used here). A number of reasons have been mentioned in literature to explain – why microbial activity decrease following N additions. The most plausible factors associated with reduction of microbial activity are (i) a drop in pH; (ii) a shift in microorganism populations; and (iii) a repression of the formation of hydroxide radicals by microorganisms (Fog, 1988). Inhibition of microbial respiration by excessively high osmotic potential (Johnson and Guenzi, 1963) and increased microbial processes that consume CO_2 (such as autotrophic nitrification) (Tate, 1977) are also mentioned as reasons. In this study, a drop in pH cannot be a reason as the change in pH over the incubation period was not bigger than 0.3 units (data not shown). Decreases in respiration rates are, therefore, mainly direct results of the increases in soil N availability (Ramirez et al., 2010) than indirect effects caused by the form of N added.

5.4.4. Microbial activity as influenced by pH

The initial pH values in Table 1 were similar to the reported data for *Sphagnum* originated peats (4.0 to 4.6) (Bohlin et al., 1989). The lower pH value in *Sphagnum* peat types is partly attributed to the presence of a relatively higher concentration of dissolved organic acids (Steinmann and Shotyk, 1997). Commercially available peat-based media are, however, often limed as most horticultural crops grow in a pH range between 5.5 and 6.5 (Reinikainen, 1997). Therefore, the rates of the intrinsic organic matter decomposition were tested at three pH ranges (pH of 4.5 to represent the natural pH condition, and 5.5 and 6.5 units to represent the lower and upper limits for optimum range) using peat samples obtained from different areas. It was found that an increase in pH from 4.5 to 5.5 considerably increased CO_2 emissions from all peat types (Table 3). Although CO_2 evolution from all peat samples was higher at pH 6.5, the relative increases in weakly humified peats were considerably higher than the strongly humified ones indicating the use of weakly humified peats at a higher pH leads to excessive decomposition of intrinsic organic matter and thereby increased shrinkage during pot plant growth. Increasing pH also found to increase denitrification activity in peats (Amha and Bohne, 2011). This might, thus, be a problem when the irrigation water has a

high pH level. A high pH of 6.0, Aendekerk (1997) reported an increase in organic matter decomposition in the weakly humified (H2) Finish peat compared to a medium humified (H5) German peat, which increases shrinkage, reduces the % of air, and leads to poor root quality of *Chamaecyparis lawsoniana* `Columnaris`. Evolved CO_2 in this experiment showed negative correlations with pH at 4.5 (r = −0.57), pH at 5.5 (r = −0.58) and pH at 6.5 (r = −0.73). The higher stability of the strongly humified peat with regard to pH and physical structure than younger peat may have been attributed to its greater buffering capacity (Maher and Prasad, 2004). In general, increasing pH could improve the bacteria growth over fungi, as fungi are typically more suited to acidic environments than bacteria (Brady and Weil, 2004). In all pH conditions, the highest CO_2 emissions were measured from the eutrophic peats although no clear trends observed in the case of other peat-forming environments.

Table 3

Evolved CO_2 from peat samples adjusted to different pH condition. Samples were incubated at 25 °C

Peat types	Country of origin	initial pH	μg CO_2 g^{-1} dry peat d^{-1}			*p*
			pH = 4.5	pH = 5.5	pH = 6.5	
1[O]	Latvia	4.62	107 ± 11[a]	289 ±24	427 ± 19	< 0.001
2[O]	Lithuania	4.42	102 ± 10	238 ±19	488 ± 24	< 0.001
3[T]	Lithuania	4.60	91 ± 18	182 ±23	457 ± 37	< 0.001
4[O]	Estonia	4.71	251 ± 26	310 ±54	555 ± 26	< 0.001
5[O]	Finland	4.28	141 ± 17	262 ±37	550 ± 19	< 0.001
6[T]	Finland	4.46	180 ± 10	292 ±20	495 ± 10	< 0.001
7[O]	Finland	4.60	184 ± 24	396 ±8	576 ± 21	< 0.001
8[O]	Latvia	4.75	145 ± 20	231 ±10	367 ± 9	< 0.001
9[M]	Ireland	4.91	53 ± 9	106 ±8	145 ± 8	< 0.001
10[T]	Lithuania	4.77	108 ± 14	205 ±16	426 ± 8	< 0.001
11[O]	Ireland	4.62	40 ± 10	112 ±30	154 ± 8	< 0.001
12[T]	Germany	4.46	99 ± 31	198 ±26	314 ± 8	< 0.001
13[O]	Estonia	4.62	110 ± 9	162 ±14	271 ± 10	< 0.001
14[T]	Sweden	4.58	81 ± 10	262 ±10	370 ± 8	< 0.001
15[M]	Germany	4.80	171 ± 31	380 ±22	526 ± 21	< 0.001
16[E]	Finland	4.29	204 ± 20	418 ±30	563 ±1 0	< 0.001
17[M]	Germany	3.91	53 ± 7	119 ±9	149 ± 8	< 0.001
18[O]	Germany	4.74	48 ± 15	109 ±7	165 ± 9	< 0.001
19[E]	Finland	4.97	216 ± 40	459 ±59	609 ± 8	< 0.001
20[T]	Estonia	5.19	39 ± 9	70 ±7	92 ± 7	< 0.001

[a]mean ± standard deviation (n = 3).
[O]oligotrophic (ombrotrophic) peat.
[E]eutrophic peat.
[M]mesotrophic peat.
[T]transitional peat.

Table 4.
Evolved CO_2 from glucose and nutrients amended peat samples. All peat samples were incubated at 25 °C and at 60% of the respective water filled pore space for 28 d

Peat sample	Evolved CO_2		Peat sample	Evolved CO_2	
	µg g^{-1} peat d^{-1}	% of control		µg g^{-1} peat d^{-1}	% of control
1^O	1017	335	11°	1036	890
2^O	1150	367	12^T	775	224
3^T	1258	539	13°	758	214
4°	1169	303	14^T	855	386
5°	1000	219	15^M	757	111
6^T	1295	242	16^E	1094	152
7°	895	161	17^M	680	410
8°	1451	476	18°	725	494
9^M	993	802	19^E	608	38
10^T	1037	266	20^T	504	552

[a]mean ± standard deviation (n = 3).
[O]oligotrophic (ombrotrophic) peat.
[E]eutrophic peat.
[M]mesotrophic peat.
[T]transitional peat.

5.4.5. Microbial activity in C and nutrients amended samples

Addition of glucose plus nutrients (*exp. iv*) caused a rapid increase in the metabolic activity of the microbial biomass in all peat samples (504 to 1451 µg CO_2 g^{-1} dry peat d^{-1}; Table 4) as compared to glucose alone (475 to 1094 µg CO_2 g^{-1} dry peat d^{-1}; Table 1) to suggest that microbial activity in these peat samples may have been limited not only by the availability of easily degradable C but also by the nutrient sources. Wheatley and Williams (1989) observed a significant increase of CO_2 after the addition of both glucose and NH_4NO_3 into peat soils compared to glucose alone. A higher microbial activity (measured as a change in mineral-N) was also measured in glucose + NH_4NO_3 treated moderately humified peat, compost and a mixture of peat:compost [3:2 *v*/*v*] compared to the respective fertilized potting media without glucose (Amha and Bohne, 2009). This suggests that microbial activity from planted peat-based media could be enhanced by the addition of readily available nutrients (a set of macro- and micro-nutrients) essential for microorganisms. Evolved CO_2 from five peat types (peat 8, 14, 16, 17, and 18) were slightly higher than the theoretical amount of CO_2 expected to be produced from added glucose (0.033 M CO_2 = 1466.66 mg CO_2 L^{-1} of peat), suggesting the presence of positive priming (accelerated decomposition of the intrinsic organic matter; Kuzyakov et al.,

2002). The amount of glucose-C remained in the other peat samples were also lower than glucose alone treated peat samples (data not shown). The highest CO_2 evolution from glucose and nutrients amended peat samples were measured at the beginning of incubation period (between day 2 and 14) and then it declined to the level of microbial respiration in glucose treated samples.

5.5. Conclusions

Evolved CO_2 from all peat types increased with increasing pH although microbial responses (as measured by CO_2) in weakly humified peats were stronger than the moderately and strongly humified ones, indicating the use of weakly humified peats beyond pH of 5.5 is not advisable as it leads to structural loss (via excessive decomposition of the intrinsic organic matter). The responses of peat samples to the added C-sources were different and did not portray clear trends. It was noted, however, that the relative glucose mineralization in the Irish, two German, one Swedish and one Estonian peat was the highest (compared to control), suggesting that microbial activity in the latter peat samples was relatively not C-limited. The increased CO_2 evolution in glucose treaded samples did, however, not confirm the presence of positive priming effect. In contrast, addition of easily decomposable C source together with a set of macro- and micro-nutrients leads to a significant depletion of glucose-C over 28 d and seems to induce accelerated intrinsic organic matter decomposition in some peat samples. The responses of microorganisms for the added cellulose were positive although the observed increases (15 to 146% of control) were considerably lower than glucose and arginine (36 to 294%) treated samples to suggest that the inclusion of cellulosic containing potting mixes (e.g. wood bark, fibers, and saw dust) into the tested peat samples may have little effect on the bio-stability of the final mixes. Cumulative CO_2 emissions in the N treated peat samples were lower than in the control (by -7.3 to -75.8%) regardless of the type of N-forms used, suggesting that decreases in respiration rates were mainly a direct result of the increase N availability than indirect effects caused by the form of N added.

6. Denitrification from the horticultural peats: effects of pH, nitrogen, carbon and moisture contents

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7. Physical, chemical and botanical characteristics of peats used in the horticultural industry

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8. General Discussion

8.1. Peats as potting media

The characteristics of peat can be explained by its degree of humification (H), dry bulk density (D_{BD}), air (A_V) and water (W_V) volumes, total pore space (P_S), ash and organic carbon contents, C/N ratio, elemental as well as the botanical composition (Bohlin et al., 1989; Clymo, 1983; Teicher et al., 1987; Tolonen, 1990). This study includes results of twenty peat samples obtained from different areas of Estonia, Finland, Germany, Ireland, Latvia, Lithuania and Sweden. These peats were classified broadly into weakly humified/fibric (H3; n=7), moderately humified/mesic (H4–H6; n=10), and strongly humified/humic (H7; n=3) peats (Grumpelt, 1991), or classified into *eutrophic* (n =2), *oligotrophic* (n = 9), *mesotrophic* (n = 3) and *transitional* (n = 6) peat types (Table 1) on the basis of the peat-forming environments (Charman et al., 1994; Shotyk, 1988; Stewart and Kantrud, 1971). The botanical composition of each peat sample was determined by identifying the predominant peat-forming plant genus (Heikurainen and Huikari, 1952) where *Sphagnum* (peat moss) identified as the major peat-forming plant genus.

8.1.1. Physical properties of the tested peat samples

Although the tested peats had substantial amounts of W_V (43 to 82% *v/v*; Table 1), their mean water buffering capacities were low (< 8% of W_V) to suggest that all peats cannot deliver enough water to the cultivated plant once the easily available water (EAW; water held between 1 and 5 kPa) is gone. However, the available water in all but six peat samples was within the range reported for quality potting media (30 to 45% *v/v*; Verdonck et al., 1983). These six peats were relatively dry at the time of delivery to suggest that laboratory determined water and air volumes (DIN EN 13041, 2000) are greatly influenced by the initial moisture contents in the samples (Amha et al., 2010; Picken and Reinikainen, 2009), as a dried peat often exhibits a hydrophobic nature (Valat et al., 1991). It was also observed that the greater percentages of water in the tested peats were held at a higher moisture tension (> 10 kPa), which is mostly unavailable for root uptake (Puustjarvi and Robertson, 1975). The weakly humified peats had the lowest mean D_{BD} and the strongly humified peats had the highest D_{BD}

(Table 1). The highest D_{BD} in the strongly humified peats was associated with the presence of secondary particles (which resulted from humification processes; Amha et al., 2010). With regard to the trophic levels, the oligotrophic peats had the lowest D_{BD} followed by the mesotrophic, transitional and eutrophic peats.

Air volume is another important index that determines the quality of growing media (Morard et al., 2000), as its poor availability in the rhizosphere has negative consequences on the plant biomass growth, root formation activity, and water and nutrient uptake. In literatures, however, there is no clear consensus about the threshold value for "enough air" and it ranged from 6–10% to 30–40% *v/v* (Bohne and Wrede, 2005 and literatures cited therein). Air contents in Table 1 seem to show a decreasing trend with increasing H-levels. Teicher et al. (1987) reported an A_v of 4–45% *v/v* for 30 *Sphagnum* derived peats, which is slightly lower than samples included here (10.4 to 52.6% *v/v*; Table 1). Wever (1991) concluded that peat substrates with air contents lower than 6% are not suitable for plant growth. It should be noted, however, that the initial air capacity might not guarantee enough air during cultivation as it decreases over time due to compaction, physical breakdown of fibers and organic matter decomposition (Nash and Laiche, 1981).

8.1.2. Chemical properties of the tested peat samples

The concentration of available K, available P, N_{min}, Mg and trace elements in horticultural peats could range from none to very low amount (Gottschall et al., 1989), which was a case in this study (Table 1). The low concentrations of mineral elements in *Sphagnum* containing peats might be explained by the presence of low concentration of P (Gibson et al., 1995), K, Mg and Ca (Malmer and Nihlgård, 1980) in the precipitation. Apart from the lowest nutrient concentrations in peats, the availability of these elements to the plant and microorganisms can also be affected by their respective elemental forms. The majority of N in peats is, for instance, found in a bound form and unavailable for plants or microorganisms (Kaunisto and Pietiläinen, 2003). Shotyk (1988) also indicated that the divalent and trivalent cations in the peats are bound strongly to OM compared to the monovalent cations. Most micro- and macro-nutrients in the tested peats decreased with increasing H-levels (Table 1). However, since the contents of N_{min}, P, K and some micronutrients were low, they could be neglected during fertilization.

Table 1.
Selected physical and chemical properties of peat samples when classified on the basis of the von Post degree of humification and the peat-forming environment

Variables	Humification degree			Peat-forming environment			
	≤H3 (n = 7)	H4–H6 (n = 10)	≥H7 (n = 3)	Eutrophic (n = 2)	Oligotrophic (n = 9)	Mesotrophic (n = 3)	Transition (n = 6)
1. Physical							
D_{BD} (g L^{-1})	45 (40 – 51)[a]	71 (44 – 98)	104 (88 – 121)	131 (121 – 141)	46 (40 – 55)	61 (49 – 67)	86 (69 – 98)
P_s (% v/v)	96 (94 – 97)	95 (92 – 97)	92 (91 – 93)	92 (89 – 95)	96 (93 – 97)	94 (92 – 96)	94 (90 – 97)
W_V (% v/v)	69 (46 – 79)	67 (43 – 82)	75 (72 – 78)	79 (77 – 80)	67 (46 – 80)	69 (43 – 82)	71 (50 – 80)
A_V (% v/v)	27 (16 – 50)	27 (10 – 53)	15 (10 – 20)	12 (11 – 14)	28 (14 – 50)	25 (10 – 53)	23 (10 – 45)
EAW (% v/v)	40 (21 – 47)	34 (24 – 47)	33 (26 – 40)	36 (33 – 39)	38 (21 – 47)	28 (24 – 35)	38 (26 – 47)
WBC (% v/v)	5.3 (2.4 – 7.4)	4.9 (2.8 – 7.4)	5.2 (3.3 – 6.9)	4.6 (2.4 – 6.6)	5.6 (4.7 – 6.7)	6.3 (4.1 – 7.4)	3.0 (2.8 – 3.3)
$S_{(\%)}$(% v/v)	33 (25 – 37)	36 (27 – 47)	42 (39 – 46)	42 (39 – 46)	32 (21 – 37)	33 (27 – 37)	42 (37 – 47)
2. Chemical							
pH	4.5 (4.3 – 4.7)	4.6 (3.9 – 4.9)	5.0 (4.7 – 5.2)	4.8 (4.6 – 5.0)	4.6 (4.3 – 4.8)	4.4 (3.9 – 4.9)	4.7 (4.5 – 5.2)
EC (mS m^{-1})	28 (21 – 33)	47 (21 – 89)	111 (37 – 185)	40 (40 – 40)	30 (21 – 41)	60 (30 – 89)	66 (27 – 185)
W_{ash} (% m/m)	1.4 (0.3 – 2.3)	2.3 (0.7 – 4.2)	6.2 (3.9 – 8.6)	6.4 (4.6 – 8.2)	1.9 (0.3 – 4.1)	2.8 (1.1 – 4.2)	2.9 (0.8 – 8.6)
N (mg g^{-1})	0.8 (0.5 – 1.0)	0.9 (0.8 – 1.1)	1.6 (1.0 – 2.1)	13 (9 – 17)	8 (5 – 10)	9 (8 – 10)	11 (7 – 21)
P (mg g^{-1})	0.4 (0.3 – 0.7)	0.4 (0.2 – 0.7)	0.5 (0.4 – 0.6)	0.3 (0.3 – 0.4)	0.2 (0.1 – 0.4)	0.3 (0.2 – 0.4)	0.3 (0.1 – 0.4)
Ca (mg g^{-1})	4.2 (3.2 – 5.4)	6.2 (4.1 – 13.3)	12.4 (4.9 – 20.0)	3.6 (3.1 – 4.1)	5.9 (3.7 – 13.3)	5.2 (4.2 – 6.3)	7.0 (3.2 – 20.0)
Mg (mg g^{-1})	0.7 (0.5 – 1.0)	1.2 (0.5 – 2.1)	1.2 (0.9 – 1.4)	0.9 (0.5 – 2.0)	1.4 (0.9 – 2.1)	0.9 (0.5 – 1.2)	0.8 (0.4 – 1.2)
K (µg g^{-1})	135 (81 – 176)	151 (70 – 366)	73 (66 – 73)	131 (82 – 181)	120 (71 – 176)	124 (69 – 220)	160 (66 – 366)
Mn (µg g^{-1})	15 (3 – 32)	17 (4 – 53)	25 (23 – 26)	37 (19 – 55)	14 (3 – 26)	15 (4 – 30)	24 (5 – 53)
Fe (mg g^{-1})	0.4 (0.2 – 0.7)	0.6 (0.2 – 1.0)	1.3 (0.4 – 2.2)	1.2 (1.2 – 1.3)	0.5 (0.2 – 0.9)	0.6 (0.2 – 1.0)	0.7 (0.3 – 2.2)

[a]mean (range; minimum – maximum value).
D_{BD} – dry bulk density.
P_S – total pore space.
W_V – water volume.
A_V – air volume.
EAW – easily available water.
WBC – water buffering capacity.
$S_{\%}$ – shrinkage percentage.
EC – electrical conductivity.
W_{ash} – ash content.

8.1.3. Relationships between physicochemical properties

Since growers and potting media producers are commonly describing peats in terms of their H-levels (von Post, 1924), studying the possible relationships between H and other physicochemical properties might help to generate a quick information about some parameters (e.g. W_V, A_V, EAW, and the water buffering capacity) whose determination take up to 10 days (DIN EN 13041, 2000). The H-values had strong correlations with D_{BD}, P_S, W_m, W_{om}, total N, total C, EC and resource availability indices (C/N, N/P, N/K) but correlated poorly with pH, W_V, A_V, WBC, and EAW elucidating its limitation to predict the later parameters. One should also be aware that the von Post humification scale (H) requires subjective evaluations that might produce data with moderate repeatability. However, D_{BD} (being an objective measurement) had similar relations with other physicochemical properties suggesting that D_{BD} could be used to describe the characteristics of horticultural peats when data on H is not available. Overall, a larger proportion of the total variance in the measured/computed physicochemical data (i.e., 42 %) was explained by factors related to peat humification (H, D_{BD}, W_{ash}, and C/N ratio) and botanical composition (Table 2). The hydrological data (W_V, A_V, W_m, and EAW) and mineral composition (total C, K, Mg, total P, Mn, C/P, and N/P) explain only about 23 % of the variance in the collected data.

Table 2.
Loadings plot for the first two principal components

Variables	Component 1	Component 2	Variables	Component 1	Component 2
H	-0.282	-	W_V	-0.132	0.307
D_{BD}	-0.256	-	A_V	0.167	-0.275
W_{ash}	0.267	-	EAW	-	0.253
EC	-0.252	-	W_m	0.187	0.216
Sph	0.270	-	$S_{(\%)}$	-	0.270
Car	-0.199	-	C	-0.107	-0.281
Bry	-0.114	-	Mg	-	-0.189
Sec	-0.233	-	P	-	0.348
Mix	-0.187	-	Mn	-	0.288
pH	-0.123	-	Fe	-0.245	0.131
N	0.283	-	C/P	-	-0.362
Ca	-0.184	-	N/P	-0.170	-0.282
C/N	-0.240	-0.104	N/K	-0.103	-0.256

Sph – *Sphagnum*; *Car* – *Carex; Bry* – *Bryales; Sec* – secondary particles formed from the humification process; mix – the mixtures of at least two of the following flora remains (*Eriophorum*, small shrubs. soft and hard wood); H – degree of humification; D_{BD} – dry bulk density; W_m – moisture content at 1 kPa as expressed in % *m/m*; P_S – total pore space; A_V – air volume; W_V – water volume; EAW – easily available water; $S_{\%}$ – shrinkage at 105 °C; EC – electrical conductivity.

8.2. Microbial activity and biomass in the tested peats

8.2.1. Methods comparison

A method that acceptably predicts the biodegradability of organic potting media (e.g. peats) is of a great importance to asses microbially mediated processes during crop cultivation. Native microbial respiration showed strong correlations with (MB-C_{SIR}; r = 0.89) and FE (MB-C_{FE}; r = 0.83) but not with FI (MB-C_{FI}; r = 0.35), AA (r = 0.61) and N-stability (r = –0.16). The computed k_{EC} value (0.436 ± 0.09) was nearly similar to the widely accepted value for soils (0.45; Sparling et al., 1990; Joergensen, 1996) to confirm that only FE and SIR can be used as reliable methods to estimate microbial biomass-C in a wide range of peat samples. Our findings were in accord with Inubushi et al. (1991) who suggested that only few methods (e.g. SIR and FE) are suitable for peat soils. In arable soils, however, all these methods resulted in very similar patterns for ranking microbial biomass-C (Kaiser et al., 1992).

8.2.2. Effects of the intrinsic organic matter

It was found that native microbial respiration over short- (10 d) and long-term (180 d) incubation period, MB-C_{SIR} and MB-C_{FE} in the tested peat samples were poorly predicted by H, %N, total OC (TOC), pH, OM content, and the C/N ratio. Microbiological data, however, showed considerably higher and positive correlations with all dissolved organic fractions (CWC, HWC, SSC, and SSC-H) to suggest that extraction of dissolved OC would provide greater insight into the labile OM pool responsible to the degradation of peat samples. This assumption seems to be supported by the computed inverse but weak relationships between H and all water- and salt-extractable OC (excluding the two eutrophic peats; r = −0.51 to −0.63). In all peats, the quantity of dissolved OC was increased from CWC to SSC-H through SSC and HWC (Table 3).

Both OC extracted in hot-water bath (HWC and SSC-H) showed strong correlations with long-term evolved CO_2 (r = 0.94 and r = 0.92, respectively). Fischer (1993) also found strong correlation between HWC contents in soils and CO_2 evolution, which would indicate that a proportion of the HWC must be easily available for microbial utilization.

Boiling samples at 70 °C reported to kill vegetative microbial cells (Sparling et al., 1998) to suggest that the procedure adopted here (boiling the samples in hot-water bath for 16 h) would make microbial biomass components extractable. However, the amounts of OC in SSC-H and HWC were 1.3 to 3.2-fold and 0.8 to 2.3-fold of the MB-C estimated by FE, respectively, indicating that boiling peat samples in a hot-water bath would also extract appreciable amounts of the OC from non-biomass organic fractions. Overall, both HWC and SSC-H can be used as indicators of microbial activity and biomass of peats. It should be noted, however, that the salt extracted OC showed less deviation within replications (i.e., a higher reproducibility) than water extracted OC.

Table 3.
Mean properties of organic C fractionates, microbial activity and biomass in peat samples

Variable	Peat-forming environment				Humification degree		
	[a]Oligo	[b]Meso	[c]Trans	[d]Eut	H3	H4–H6	H7
TOC	[e]494±17	503±14	496±7	490±30	485±8	499±14	512±10
CWC	121±46	96±47	102±28	179±10	130±37	114±40	100±74
HWC	1328±651	1047±703	1247±610	2855±372	1623±595	1290±650	1341±1544
SSC	717±222	507±222	678±170	894±112	802±184	657±201	549±239
SSC-H	1883±715	1359±845	1614±670	3122±301	2175±625	1665±704	1685±1447
PMN	240±48	324±97	290±64	387±11	274±51	284±90	295±77
CO_2	217±97	173±112	217±87	421±20	276±65	211±107	191±189
$MB\text{-}C_{FE}$	1037±577	672±262	838±382	1594±207	1288±547	788±303	890±740
$MB\text{-}C_{SIR}$	1025±409	760±531	1055±527	1518±190	1221±473	1015±405	725±583
$F\text{–}CO_2$	50±14	36±2	30±7	75±4	53±15	38±16	46±23
$B\text{–}CO_2$	11±18	44±6	32±15	8±0	10±15	27±18	36±25
IAR	0.96±0.16	1.36±0.10	1.19±0.15	1.01±0.1	0.99±0.17	1.17±0.21	1.12±0.14
F:B	3.1±2.9	0.8±0.1	1.9±2.6	9.8±0.1	5.7±2.9	2.3±3.0	3.7±5.3

TOC – Total OC in mg g^{-1} dry peat.
CWC – Cold-water extractable OC in μg g^{-1} dry peat.
SSC – Salt-soluble OC in μg g^{-1} dry peat.
HWC – Hot-water extractable OC in μg g^{-1} dry peat.
SSC-H – Salt-extractable OC in the hot-water bath in μg g^{-1} dry peat.
PMN – potentially mineralizable N in μg g^{-1} dry peat.
CO_2 – mean daily evolved CO_2 over six months in μg g^{-1} dry peat.
$B\text{–}CO_2$ – Bacterial respiration (% of control).
$F\text{–}CO_2$ – Fungi respiration (% of control).
IAR – Inhibition Additivity Ratio.
F:B – Fungal-to-bacterial ratio.
[a]Oligotrophic (Ombrotrophic).
[b]Mesotrophic.
[c]Transition.
[d]Eutrophic.
[e]mean ± standard error.

8.2.3. Effects of microbial population

The relationship between CO_2 evolution and the size of microbial biomass is significantly affected by the fungal-to-bacterial biomass ratio (Sakamoto and Oba, 1994). Streptomycin addition into peat samples inhibited microbial respiration by 5.5 to 53.5% while cycloheximide addition by 18.9 to 78.0%. In case of three oligotrophic peats, however, an increase in SIR was observed to suggest that streptomycin might have been used as C source in these peat samples. Addition of both antibiotics as a mixture did not also result in complete inhibition of the substrate-induced respiration rate (only by 39.9 to 85.6%). The calculated fungal-to-bacterial ratio and inhibitor additivity ratio (IAR) varied considerably between peat samples, and ranged from 0.46 to 9.96 and 0.76 to 1.48, respectively. The F:B ratio tends to decrease with increasing humification degree (Table 3). The relative proportions of fungal and bacterial activity were compressed to fit an IAR of 1.0, showing both overlapping and antagonistic effects (Chapter 4, Fig.3) to suggest the need for antibiotics optimization for each peat types. Overall, the contributions of fungi were mostly higher in the oligotrophic and eutrophic peats while bacterial dominance was higher in the transitional and mesotrophic peat samples. Since fungi and bacteria have different assimilation-to-respiration ratio, the observed differences in microbial population would have consequences on C- and N-cycling in their respective peat-forming environments.

8.2.4. Effects of additives (lime, fertilizer and carbon)

8.2.4.1. CO_2 evolution

Addition of glucose into all peat samples stimulated CO_2 production by 78 to 514% of control. However, the rates of CO_2 evolution differed considerably between peat samples (Fig. 1a). For instance, in peat 1, the maximum relative increase of CO_2 (with respect to control treatment) was observed 1 d after glucose addition but it decreased rapidly over the next 8 days and stabilized thereafter. The maximum CO_2 increase from peat 18 was observed between day 4 and 7 but decreased sharply until day 14 where it stabilized. In peat 16, evolution of CO_2 in glucose amended samples reached maximum between day 1 and 3 but dropped sharply on the next day. The shape of the curves in the other peat

types were represented by one of the above three patterns although the relative increases differed considerably between peat types. It appeared that peat 6, 7, 10, 15, and 19 shared a similar pattern with peat 1, and peat 2, 3, 4, 5, 12, and 13 shared similar patterns with peat 18. Peat sample 8, 9, 10, 11, 14, and 17 showed similar patterns as of peat 10. However, the glucose mineralization curve in peat 20 reached its maxima on day 7 and slightly decreased thereafter. In all cases, it appeared that addition of glucose induced a higher CO_2 evolution in the first few days and no lag period was observed (Fig 1a). De Nobili et al. (2001) and Hamer and Marschner (2002) also observed a decreasing additional C mineralization from glucose-amended samples after 8 days. In contrast, a lag period of 2 to 3 d was reported by Turner and Carlile (1983) when peat-based media were enriched with glucose. Such differences might be due to the differential incubation temperature and samples equilibration (11.5 °C and no equilibration vs. 25 °C and equilibration used here). After such lag period, however, they measured maximum increases in CO_2 evolution (up to 100 mg CO_2-C per L^{-1} of peat d^{-1}) between day 3 and 8.

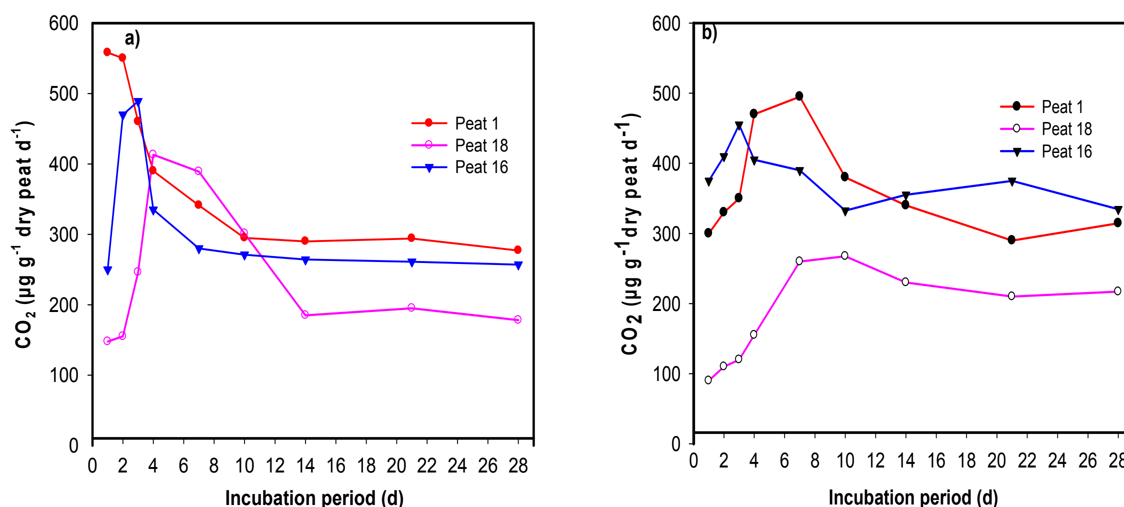

Fig. 1. CO_2-evolution (µg g^{-1} dry peat d^{-1}) after the addition of glucose (a) and arginine (b).

The mean daily CO_2 evolution from all peat samples increased after arginine addition in the range of 36 to 294% compared to control. Arginine metabolisms in most peats also showed three distinct patterns (Fig. 1b): (i) evolution of CO_2 increased considerably until day 7 but decreased between day 10 and 21. A spike of CO_2 evolution was then observed on day 28 (e.g., peat 1, 2, 3, 4, 6, 8, and 12), (ii) CO_2 evolution increased until day 10 and declined until day 21 (e.g., peat 9, 11, 17, 18, and 20), and (iii) mostly a

zigzag pattern after its pick on day 7 (e.g. peat 7, 13, 15, 16, and 19). However, peat 5 and 10 showed very steady increases in CO_2 over the incubation period (data not shown).

The rates of CO_2 increases between cellulose treated and untreated peats were similar until day 4, showing the presence of lag period after cellulose additions (data not shown). However, CO_2 evolution in all peat types started to increase in the latter incubation periods but with different rates. Cumulative CO_2 emissions in the N treated peat samples were lower than in the control (by -7.3 to -75.8%) regardless of the type of N-forms used, suggesting that decreases in respiration rates were mainly a direct result of the increase N availability than indirect effects caused by the form of N added (see Chapter 5). The maximum CO_2 evolution was also measured at about 60% water filled pore space, whereby its amount decreased considerably for increased moisture contents (Fig. 3a). Davidson et al. (2000) also found optimum water content for microbial respiration at intermediate ranges, where respiration decreases at water contents either higher or lower than the optimum. They stated that optimum water content is usually somewhere near field capacity.

8.2.4.2. (N_2O+N_2)-N emission

The production and emission of N_2O/N_2 via denitrification can be influenced by several soil and environmental factors including the availability of NO_3^-–N (Matson and Vitousek, 1990), readily-oxidizable organic substances (Duxbury et al., 1982), the water content (Klemedtsson et al., 1991), pH (Brady and Weil, 1999), temperature (Saad and Conrad, 1993), oxygen availability and the redox potential (Davidson et al., 1993; Vymazal, 2007). Basal and potential denitrification rates from the unlimed peat samples varied widely from 2.0 to 21.8 and from 118.9 to 306.6 µg (N_2O+N_2)-N L^{-1} dry peat h^{-1}, respectively, with the highest rates from the eutrophic peat and the lowest from the transitional one (details were reported in Amha and Bohne, 2011). Increasing the initial pH of the bulk samples from 4.3–4.8 to 5.9–6.5 increased (N_2O+N_2)-N emissions by 3.6–14 fold (in C and N unamended samples) or by 1.4–2.3 fold (in C- and N-treated peat samples) to confirm that the activity of denitrifying microorganisms was limited by low pH.

Emissions of (N_2O+N_2)-N from oligotrophic, mesotrophic and transitional peats were markedly increased by the addition of 0.15 g NO_3^-–N L^{-1} dry peat but further additions had no effect. However, the mean daily emissions of (N_2O+N_2)-N from the eutrophic peat decreased markedly with increasing NO_3^-–N concentrations. Although a decrease in (N_2O+N_2)-N was not expected with increasing NO_3^-–N, high NO_3^- concentration in soil/pure culture reported to have an inhibitory effect on denitrification by inhibiting the activity of N_2O reductase (Gaskell et al., 1981) and NO_2^- and NO reductase (Payne and Riley, 1969). The addition of easily decomposable C source could markedly increase the active denitrifiers biomass and thereby increases (N_2O+N_2)-N emissions (Myrold and Tiedje, 1985). Denitrification rates were increased by increasing glucose concentration suggesting that the activity of denitrifiers in all peat types was limited by the low availability of easily decomposable C source.

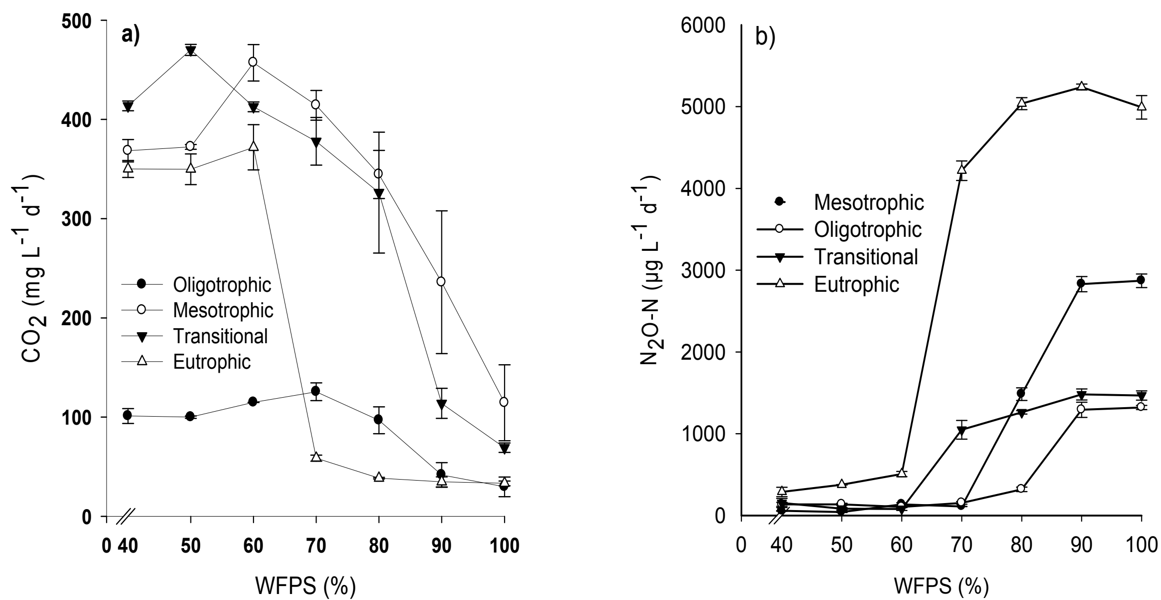

Fig. 3. Emissions of (a) CO_2 and (b) (N_2O+N_2)-N from samples treated with 0.4 g glucose-C and 0.4 g NO_3^-–N L^{-1} dry peat but differing in incubation moisture contents. Each point was the mean of several measurements and the vertical bars indicate standard errors

Since the moisture contents influence the availability and diffusion rate of oxygen in peat substrates (Agner and Schenk, 2005), the effect of moisture (40–100% WFPS) on the production of (N_2O+N_2)-N was studied under laboratory conditions. Increasing moisture contents of all peats from 40 to 50% WFPS did not significantly ($p > 0.05$) increase (N_2O+N_2)-N emissions (Fig. 3b). However, a positive effect was observed when

the moisture contents were increased from 60 to 70% WFPS in the eutrophic peat, from 70 to 80% in the transitional, from 80 to 90% in the oligotrophic and from 70 to 90% in the mesotrophic peat. A moisture content of 60% WFPS (≈ 56 % *v*/*v*) was generally found to be a critical threshold limit in all peat types above which the emission of (N_2O+N_2)-N increased considerably. Denitrification rates are reported to increase with increasing water contents, especially when WFPS exceeds 60% (Groffman and Tiedje, 1991). It can be concluded that liming, N-fertilization, availability of easily decomposable C and moist condition above 60% WFPS could encourage denitrification from peats although the rates are greatly influenced by the peat-forming environments (eutrophic > mesotrophic > oligotrophic > transitional types). We should also realize that the initial water-air dynamics in these peats might be changed during crop cultivation (due to settling and organic matter decomposition), which decrease the availability of oxygen and thereby increase N_2O emission.

Grouping of peat samples were made using data from the mean daily CO_2 evolution over six months, microbial biomass-C estimated by the fumigation extraction and substrate-induced respiration methods, total OC, cold-water extractable C, hot-water extractable C and salt-extractable C before and after fumigation (Chapter 3; Fig. 5). The hierarchical cluster analysis was established according to the Ward method based on the Euclidean square distance; and the tested peats were clustered into three distinct groups whereby peats from Ireland and 2 of the 4 German peats produced the lowest CO_2 (group ii), peats from the Baltic States occupied the middle ranges (group iii), and most peats from Finland produced the highest CO_2 (group i). This excessive decomposition of OM from the Finland's peat might suggest that these peats could not be used in long-term plant production, as it leads to the loss of structural stability. However, stable potting media can be formulated from such peats if they are mixed in the right proportion with others (e.g. with group ii). Prasad and O'Shea (1997) also found differences in CO_2 evolution between peats from two origins where the Irish peat showed strong microbial stability and the Baltic States showed the opposite.

9. Summary

The general objective of this thesis work was to investigate the microbial activity and biomass of peats in relation to their intrinsic OM composition, pH, moisture, and C and N inputs as this information on a large set of peat samples is largely missing from published literature. These peat samples (n = 20) were obtained from different areas of Finland, Germany, Ireland, Sweden, and the Baltic States (Estonia, Lithuania and Latvia). The following points can be summarized:

Peat properties

- The properties of peat cannot be explained by a single variable. Instead, factors related to peat humification (bulk density, ash content, C/N) and botanical composition explained much of the total variations (~42%).

- The tested peats had substantial amounts of water volume (W_V) but their respective mean water buffering capacities were low (<8 % of W_V), indicating these peats cannot deliver enough water to the cultivated plant once the easily available water (i.e., water held between 1 and 5 kPa) is gone.

- The initial moisture contents in peats had significant effects on the water and air volumes determined at laboratory conditions.

- The mineral contents (NH_4^+–N, NO_3^-–N, P, Mg, K, Ca, Mn and Fe) in most peats were considerably low and could be neglected during fertilization.

- Basal/native respiration in the tested peat samples was not influenced by %N, pH, organic matter content, and the C/N ratio.

- *Sphagnum imbricatum* containing peat samples produced the lowest CO_2-C and *S. angustifolium* dominating peats the highest CO_2-C.

Organic matter composition

- The amounts of salt-extracted OC in hot-water bath (SSC-H) were 10.6 to 21.0 times higher than that extracted by cold-water. SSC-H also equivalent to 107–198% of hot-water extracted OC (HWC), 131–409% of salt-extracted OC, and 0.13–0.65% of total OC.

- OC extracted in hot-water bath (HWC and SSC-H) showed strong correlations with long-term evolved CO_2 and MB-C suggesting both can be used as indicators of microbial activity and biomass of peats. However, SSC-H showed less deviation within replications than HWC to suggest the higher reproducibility in SSC-H data.

- The eutrophic peats contained the highest mean dissolved OC followed by the oligotrophic, transitional and mesotrophic peat types.

- There was a strong linear relationship (r = 0.94) between the mean daily CO_2 evolution over 10 d and mean daily CO_2 evolution over 180 d, indicating microbial activity measured after 10 d is a surrogate to long-term OM degradation in peats.

Responses to additives

- A moisture content of 60% WFPS (≈56 % v/v) was found to be a critical threshold limit in all peat types above which the emission of (N_2O+N_2)-N increased but CO_2 evolution decreased. The emissions were mostly in the following order: eutrophic > mesotrophic > oligotrophic > transitional peat.

- Cumulative CO_2 emissions in the N treated peat samples were lower than in the control regardless of the type of N-forms used, suggesting that decreases in respiration rates were mainly a direct result of the increase N availability than indirect effects (e.g. change in pH) caused by the form of N added. Similarly, denitrification rates were not enhanced by N additions beyond 0.15 g L^{-1} dry peat.

- Addition of glucose into all peat samples markedly increased CO_2 production over 28 d but it did not confirm the presence of positive priming effect (accelerated intrinsic organic matter decomposition). The responses of microorganisms for the added

cellulose were positive although the observed increases were considerably lower than glucose- and arginine-treated samples indicating that the inclusion of cellulosic containing potting mixes (e.g. wood bark, fibers, and sawdust) into the tested peat samples may have little effect on the bio-stability of the final mixes.

- The rates of (N_2O+N_2)-N and CO_2 emissions considerably increased with increasing pH. The effects were more pronounced in the weakly humified peats. The use of these peats at higher pH (> 5.5), therefore, leads to significant decomposition of OM and causes shrinkage, which in turn reduces availability of air to plant root.

Methods and microbial population

- FE and SIR methods are reliable methods to estimate microbial biomass-C in a wide range of peat samples but not the AA, FI and N-stability methods.

- Additions of 3 mg streptomycin and 4.5 mg cycloheximide g^{-1} moist peat (adjusted at 60% water filled pore space) were sufficient to inhibit substrate-induced bacterial and fungal respiration, respectively.

- The calculated fungal-to-bacterial ratio and the inhibitor additivity ratio (IAR) varied considerably between peat samples, and ranged from 0.46 to 9.96 and 0.76 to 1.48, respectively. A slightly wider range of IAR emphasizes the need for antibiotics optimization step for each peat types to circumvent their effects on non-target organisms. The higher fungal contributions in the eutrophic and oligotrophic peats compared to the mesotrophic and transitional peat types suggesting the structure of microbial community in peats is greatly influenced by the peat-forming environments.

Overall, the tested peat samples were clustered into three distinct groups whereby peats from Ireland and Germany produced the lowest CO_2 and peats from Finland occupied the highest ranges. However, most peats from the Baltic States produced CO_2 occupying the middle range. Excessive decomposition of OM in most Finish peats might have unintended consequences if these peats are to be used for the production of long-term pot plants. Therefore, they have to be mixed with less decomposable peats to maintain their stability during a long-term crop production.

10. References

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Curriculum Vitae

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1999-2002	BSc in Forestry	Debub University, WGCF, Ethiopia	Great distinction
2004-2006	MSc in Horticulture	L. University of Hannover, Germany	Summa cum laude
2007-2011	PhD	L. University of Hannover, Germany	Summa cum laude

Professional Experience

- January 2003 to August 2004: Junior researcher at Ethiopian Agricultural Research Organization, Jimma, Ethiopia
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