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Miscanthus as primary feedstock for growing media in soilless cultivation

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Ask, and it will be given to you,
Seek, and you will find
Knock, and the door will be opened to you.
(Matthew 7, 7)

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Summary

To meet the increasing demand for soilless substrates in the context of limited availability of peat and other key substrate components, the substrate industry needs more and diverse renewable materials. Dry biomass of miscanthus crop (*Miscanthus* spp.) is a promising feedstock for soilless substrates in the temperate regions because of its high biomass production under low-input and ecosystem services. The objectives of this PhD work were (i) to evaluate potential supply capacity of miscanthus feedstock, opportunities and challenges when developing miscanthus into a commercial substrate, and (ii) to optimize substrate performance including increasing water holding capacity, manipulating substrate pH within the optimal range for greenhouse crops and reducing nitrogen (N) immobilization, using the "stand-alone substrate" approach.

The first objective is presented in chapter 2 as a literature review. Given potential large land available for energy crops and relatively small market for miscanthus in Europe, potential supply capacity of miscanthus was estimated to be large with less intense competition for the feedstock from other industrial activities. Besides efforts to improve substrate performance, future studies should investigate cascade utilization of used miscanthus substrate. This would not only increase resource efficiency and by that sustainability, but also add additional market opportunities to reduce competition for the feedstock.

In chapter 3, miscanthus performance as growbag substrate for soilless cultivation of tomatoes under practical conditions with cascade utilization as direct combustion was assessed. The treatments included miscanthus genotypes, substrate processing and substrate amendment with calcium nitrate ($Ca(NO_3)_2$) or elemental sulfur (S^0). Results showed that all miscanthus treatments did not reduce substrate pH nor increase nitrate concentration in root-zone solution compared to those in stone wool and coir. Plants in all miscanthus substrates produced similar fruit yield to those in stone wool, but lower than those in coir. Blossom-end rot (BER) fruit mainly caused yield loss, which were lowest for coir and highest for stone wool, with miscanthus intermediate. Further modifications concerning crop management and substrate properties could reduce BER incidence. Combustion quality of used miscanthus substrates were comparable to new materials, which demonstrates clearly the opportunity of cascade utilization.

In chapter 4, the effects of primary mechanical processing on substrate morphology, hydrological properties, pH and N immobilization were investigated. Mechanical processing altered substrate particle size and shape and consequently changed substrate water holding capacity and wettability, but hardly changed substrate pH and N immobilization. To enhance nutrient availability in miscanthus substrate, further studies should focus not only on increasing nitrate concentration, but also on increasing calcium and reducing ammonium, potassium and phosphorus concentration in substrate solution at cultivation.

In chapter 5, the effects of washing and steaming pretreatments on reducing N immobilization in miscanthus substrate were assessed. Washing and steaming treatments did not reduce N immobilization in miscanthus. Steaming without pretreatment showed mild negative effect as delayed germination rate

of Chinese cabbage and short lateral root length. However, washing could be a promising treatment as substrate solution showed higher calcium and lower ammonium, potassium and phosphorus concentration. At the early growth stage, Chinese cabbage seedlings in washed substrate produced longer lateral root length than those in non-treated substrate.

In summary, the investigations showed that miscanthus is a promising primary feedstock for soilless cultivation in horticultural production. The approach to use miscanthus as "stand-alone" substrate showed clearly how substrate properties could be modified to optimize its performance. Moreover, in this way miscanthus could be used in a cascading manner to increase resource efficiency through tailored uses like combustion to achieve a more sustainable horticultural production.

Zusammenfassung

Im Zusammenhang mit dem steigenden Bedarf an Substraten für den erdelosen Anbau und der stärker limitierten Nutzung von Torf und weiterer Substratkomponenten, sucht die die Praxis nach alternativen Substratrohstoffen. Miscanthus (*Miscanthus* spp.) ist als Low-Input Kultur, der hohen Biomasseproduktion und den mit dem Anbau verbundenen Ökosystemaren Dienstleistungen, ein vielversprechender Rohstoff für erdelose Substrate in den gemäßigten Regionen. Die Ziele dieser Arbeit waren (i) die Untersuchung der Rohstofflieferkette sowie deren Chancen und Herausforderungen bei der Entwicklung von Pflanzsubstraten für die Praxis; (ii) die Optimierung der Substrateigenschaften hinsichtlich der Wasserhaltekapazität, pH-Wert-Anpassung für den Anbau von Kulturpflanzen im Gewächshaus und der Möglichkeiten die Stickstoffimmobilisierung zu reduzieren. Diese Untersuchungen erfolgten unter der Prämisse Miscanthus als Pflanzsubstrat ohne weitere Komponenten zu entwickeln („stand alone“).

Die Untersuchungen zur Rohstofflieferkette (Kapitel 2) ist ein umfassender Literaturüberblick. Auf Basis der möglichen Anbauflächen für Energie- und Biomassepflanzen und der möglichen Märkte in Europa, konnte das Rohstoffpotenzial als groß bewertet werden, weil in diesem Fall die Konkurrenz zu anderen industriellen Nutzungen eher gering ist. Die Verbesserung der Rohstoffeigenschaften und die Möglichkeit zur Kaskadennutzung von Miscanthus als Einzelsubstrat würde zudem das Marktpotenzial steigern und die Konkurrenz zu anderen Märkten zusätzlich verringern.

In Kapitel 3 werden dann die Eigenschaften und Leistung von Miscanthus als Pflanzsubstrat für „Grow Bags“ in der erdelosen Kultur von Tomaten unter Praxisbedingungen und der möglichen Kaskadennutzung als Brennstoff diskutiert. Hierbei wurden sowohl unterschiedliche Genotypen von Miscanthus, verschiedene Aufbereitungsformen und Zuschlagstoffe wie Kalziumnitrat oder elementarer Schwefel betrachtet. Die Ergebnisse zeigten, dass in allen Miscanthusvarianten unter gleichen Kulturbedingungen der pH-Wert nicht sank oder der Nitratgehalt in der Wurzelzone anstieg, wie es für Steinwolle oder Kokosfasern als Vergleichssubstrate beobachtet werden konnte. Dabei waren in allen Miscanthussubstraten die Erträge vergleichbar zu Steinwolle oder Kokosfasern. In diesen Versuchen führte Blütenendfäule (BER) zu Ertragsverlusten, wobei der Ausfall bei Kokosfasern am geringsten, bei Steinwolle am höchsten und bei Miscanthus dazwischen war. Weitere Anpassungen hinsichtlich Kulturführung und Substrateigenschaften könnten den Ausfall durch BER verringern. Die Brennwerteigenschaften von Miscanthus wurden durch die Nutzung als Pflanzsubstrat im Vergleich zu ungenutztem Miscanthus nicht verändert und zeigen die Möglichkeit der Kaskadennutzung.

Kapitel 4 widmet sich im Detail den Partikeleigenschaften von Miscanthus und wie diese durch die Verarbeitung beeinflusst werden. Die mechanische Bearbeitung verändert die Partikelgröße und –form, wodurch die Substrateigenschaften hinsichtlich Wasserhaltekapazität und Benetzbarkeit beeinflusst wurden, aber nicht der pH-Wert oder die Stickstoffimmobilisierung im Substrat. Zur Optimierung der Nährstoffverfügbarkeit in Miscanthussubstraten sollte zukünftig aber nicht nur der Nitrat- oder

Kalziumverfügbarkeit, sondern auch die Ammonium-, Kalium- und Phosphatgehalte in der Nährstofflösung während der Kulturführung an das Substrat angepasst werden.

Schließlich werden in Kapitel 5 die Effekte einer Vorbehandlung von Miscanthus durch Waschen und Dämpfen auf die Stickstoffimmobilisierung untersucht. Es zeigte sich, dass weder Waschen noch Dämpfen einen wesentlichen Einfluss haben. Dämpfen alleine führte zu einer geringen Verzögerung der Keimrate von Chinakohl und leicht reduzierter Wurzellänge. Waschen dagegen erhöhte den Kalziumgehalt bei gleichzeitig verringerten Ammonium-, Kalium- und Phosphatgehalten und scheint eine vielversprechende Möglichkeit zur Optimierung der Substrateigenschaften. Zudem war bei Chinakohl die Wurzellänge im Keimlingsstadium gegenüber unbehandelten Substraten erhöht.

Die Untersuchungen zeigten insgesamt, dass Miscanthus ein vielversprechender Primärrohstoff für Pflanzsubstrate insbesondere für erdelose Kulturverfahren ist. Der verfolgte Forschungsansatz Miscanthus nicht in Mischungen mit anderen Substratrohstoffen zu untersuchen, zeigte deutlich wie und wo die Substrateigenschaften verbessert werden können. Zudem ermöglicht die alleinige Nutzung von Miscanthus eine Kaskadennutzung und erhöht so die Ressourceneffizienz und so auch eine nachhaltigere gartenbauliche Produktion.

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Abbreviations

AFP	air-filled porosity
b/l	aspect ratio
BER	blossom-end rot
C	carbon
C15, C10, C5, C3	*Miscanthus* x *giganteus* hammermilled through screen size of 15, 10, 5 or 3 mm
$Ca(NO_3)_2$	calcium nitrate
CEC	cation exchange capacity
Conv	convexity
d10, d50, d90	10^{th}, 50^{th}, 90^{th} percentile
DAT	days after transplanting
EC	electrical conductivity
FC	*Miscanthus* x *giganteus* harvested from the field
GigC15	*Miscanthus* x *giganteus* hammermilled through a 15-cm screen
GigS5	*Miscanthus* x *giganteus* shredded through a mechanical fraying facility
IMC	initial moisture content
M	*Miscanthus* x *giganteus* hammermilled through a 15-cm screen, non-treated
Mps	*Miscanthus* x *giganteus* hammermilled through a 15-cm screen, primed-steaming
Ms	*Miscanthus* x *giganteus* hammermilled through a 15-cm screen, steaming
Mw	*Miscanthus* x *giganteus* hammermilled through a 15-cm screen, washing
Mws	*M.* x *giganteus* hammermilled through a 15-cm screen, washed-steaming
N	nitrogen
NDI	N drawdown index
RobC15	*Miscanthus 'Robustus'* 24 hammermilled through a 15-cm screen
S^0	elemental sulfur
S5	*Miscanthus* x *giganteus* shredded through a mechanical fraying facility
SinC15	*Miscanthus sinensis* 21 hammermilled through a 15-cm screen
SPHT	sphericity
Sv	specific surface
SWOT	strengths, weaknesses, opportunities, threats
Symm	symmetry
U3	non-uniformity
WHC	water holding capacity
x_{cmin}	particle width, shortest chord diameter
x_{Femax}	particle length, longest Feret diameter

Chapter 1
General introduction

Due to the increasing demand for soilless substrates together with reduced availability and uncertainty of peat use in many countries across Europe, the substrate industry is searching for new alternative raw materials (Blok et al., 2021; Bragg, 2012; Röse, 2020). Any suitable growing medium or substrate should have reliable and consistent performance, large supply capacity with affordability and low environmental impacts (Barrett et al., 2016; Schmilewski, 2012).

Although being investigated as soilless substrates to a lesser extent compared to waste stream materials, renewable primary materials are potentially important, mainly for the substrate manufacturers for better management of feedstock availability and quality. Among the primary materials have been investigated, dry biomass of *Miscanthus* spp. represents one of the most promising material due to its highest straw biomass, regional availability in the temperate regions and ecosystem services (Emmerling & Pude, 2017; Heaton et al., 2010; Lewandowski et al., 2000). Miscanthus straw has been used as substrate component for nursery shrubs, bedding plants, strawberries, wood cuttings and as stand-alone substrate in growbags for tomatoes and cucumbers (Altland, 2010; Altland & Locke, 2011; Bąbelewski & Pancerz, 2018; Cárthaigh et al., 1997; Debode et al., 2018; Jensen et al., 2001; Kraska et al., 2018; Kraska & Pude, 2019).

To develop miscanthus into a commercial substrate, *two main challenges* are (i) to evaluate potential supply capacity of miscanthus feedstock, opportunities and challenges when developing this primary material into a commercial substrate; and (ii) to optimize substrate performance including increasing water holding capacity, manipulating substrate pH within the optimal range for greenhouse crops and reducing nitrogen immobilization.

In the studies on miscanthus substrates, miscanthus has been blended with peat or other commonly used substrates (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012). The blending approach has two goals including reducing the proportion of peat or other key substrate constituents in the substrates and remedying the performance limitations of tested materials (Schmilewski, 2012). While this approach is highly effective to develop substrates with direct practical importance for horticultural production, it is limiting insights into the performance of the single feedstock. Effects of single substrate constituents on the performance of a substrate mixture might not be easily distinguished, particularly when the understanding of new materials is limited.

In this PhD work, in order to study the performance of miscanthus substrate, the ‘stand-alone substrate’ approach was used. By using miscanthus as sole substrate component and focusing on feasible modifications on material itself, this work aimed to develop a profile on the sole use of miscanthus as a substrate, which is useful both in developing further modifications and in formulating 100% miscanthus substrates in the future if needed. Additionally, in the bioeconomy context, cascade utilization of used miscanthus substrate could be tailored more easily when stand-alone miscanthus substrate serves as feedstock, because feedstock quality depends only on one sole source.

The objectives of this PhD work were (i) to assess the quality of miscanthus as primary feedstock and the performance of miscanthus substrate, to estimate potential supply capacity of miscanthus feedstock and to analyze strengths, weaknesses, opportunities and threats (SWOT) when developing miscanthus into commercial substrate by conducting a literature review; (ii) to optimize the performance of miscanthus substrate by conducting trials using the 'stand-alone substrate' approach.

1. Growing media in soilless culture

Soilless culture is the cultivation of plants in systems without soil *in situ* (Gruda, 2009). It encompasses a wide range of cultivation techniques which can generally be divided into water culture and substrate-based culture. To avoid confusion, the term 'hydroponics' is not used here as its definition means water culture in Europe, but includes both water culture and substrate-based culture in the US (Blok et al., 2021; Walters et al., 2020). In water culture, plant roots directly come in contact with nutrient solution either in a static mode (the floating system) or in a dynamic mode (the nutrient film technique or the aeroponics) (Savvas & Gruda, 2018). In substrate-based culture, plant roots grow in porous rooting medium termed 'growing medium' or 'substrate'. While water culture techniques are mainly applied to grow leafy vegetables and herbs, substrate-based culture systems have a wider range of application from propagation substrates for vegetable transplants to growing substrates for soft fruits like strawberries, and fruit vegetables such as tomatoes, cucumbers and peppers (Walters et al., 2020). This PhD work focuses on substrate-based culture.

Soilless culture has gained interest for its commercial applications in the last decades (Fields et al., 2021; Schmilewski, 2017; Walters et al., 2020). Greenhouse vegetable production in the Netherlands increased 40% between 2000 and 2017, with the production in 2017 of nearly 1,775 million kg from 5,000 ha cultivation area (CBS, 2018). In Germany, cultivation area in greenhouse for tomatoes, cucumbers and peppers increased from 584 ha in 2015 to 714 ha in 2020 and even more for strawberries from 639 ha to 1512 ha (Destatis, 2021) (Figure 1.1). The advantages of soilless culture over soil-based culture are lower risk of soil-borne pathogen (Raviv & Lieth, 2008) and resource use efficiency (higher yield per unit of water and fertilizer applied) (Grunert et al., 2020; Jovicich et al., 2007).

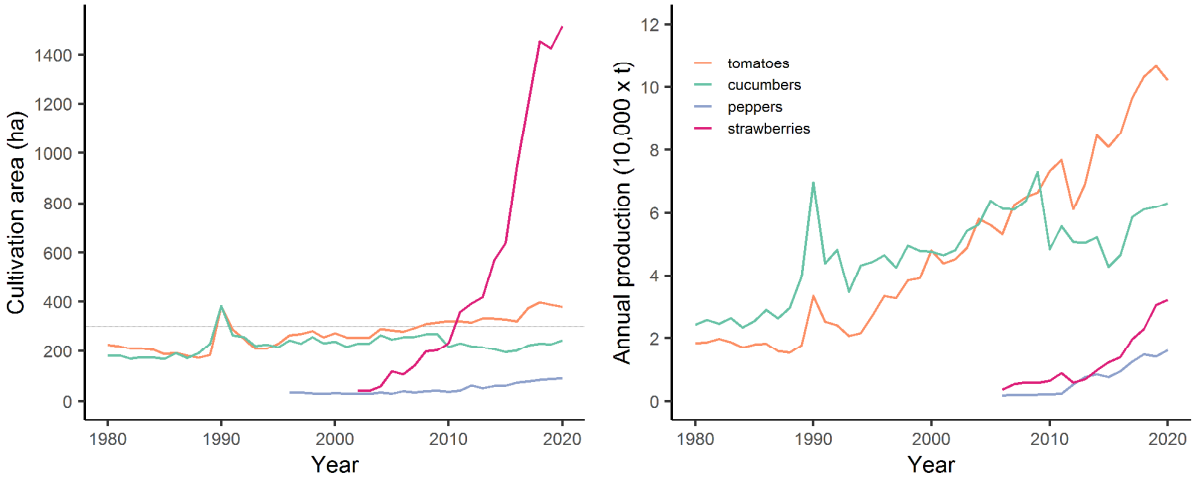

Figure 1.1 Cultivation area and production of important greenhouse vegetable crops and strawberries (including cultivation under tunnel) in Germany (1980-2020) (adapted from Destatis, 2021)

As soil replacement, growing medium is a key factor in successful crop cultivation. More than just a physical support for the plants, given a limited volume for root growth in containers, substrates must be effective in capturing adequate irrigation solution, maintaining a buffer medium in between irrigation events and supplying sufficient amount of air and nutrients to plant roots. Moreover, soilless substrates

must be free from phytotoxic substances, weeds and plant pathogens. Soilless substrates are generally characterized by the low bulk density (0.06-0.20 g cm^{-3}) and large pore volume (65-95%, v/v) (Fonteno & Harden, 2003). Water and air are captured in the pore space, which are expressed as total porosity, water holding capacity and air-filled porosity (Fonteno & Harden, 2003; Handreck, 2011). While the pore size distribution represents substrate capacity to hold and release water and air, wettability describes how easily water can spread over the surface of substrates during drying and rewetting cycles (Michel et al., 2001). Chemical composition and particle surface characteristics determine substrate pH, pH buffering capacity, electrical conductivity (EC), cation exchange capacity (CEC), nitrogen immobilization, thus control nutrient availability in root-zone solution (Bunt, 1988; Handreck, 2011). Substrate properties are dynamic parameters, which could change at sampling, at potting and during cultivation. Substrates, therefore, should be assessed under practical growing conditions (Barrett et al., 2016).

2. The need for new renewable soilless substrates

Peat is the most predominant substrate in soilless culture since 1950s, with a recent total annual volume of 26 Mm^3 accounting for 75% of total growing media production in Europe (Schmilewski, 2017). Until late 1980s, the movement towards peat reduction started because of the environmental concerns caused by peat harvesting on peatlands, e.g. carbon release from peatlands, biodiversity destruction, impacts on water cycle etc. (Bragg, 2012). The substrate industry and the policy makers have still been debating the peat reduction strategy in horticulture section (Röse, 2020). Recently, based on the increasing production of ornamental and food crops in soilless culture systems, global demand for growing media in 2050 is projected to exceed its recent volume by four times (Blok et al., 2021) (Figure 1.2). Considering the potential supply capacity of commonly used substrates, the market still needs about 65 Mm^3 volume of new substrates (Blok et al., 2021). All these requires a set of measures for the substrate industry including maintaining their production on renewable raw materials, investing into production techniques which allow less substrate used or no substrate at all and searching for new renewable materials (Röse, 2019).

Any suitable growing medium or substrate should fulfil three requirements including reliable and consistent performance, sufficient availability with affordability and minimal environmental impacts (Barrett et al., 2016; Gruda, 2019; Schmilewski, 2012). While many materials have been investigated as renewable feedstock for soilless substrates, few of them have been adopted into the market (Barrett et al., 2016; Blok et al., 2021; Gruda, 2019; Schmilewski, 2017).

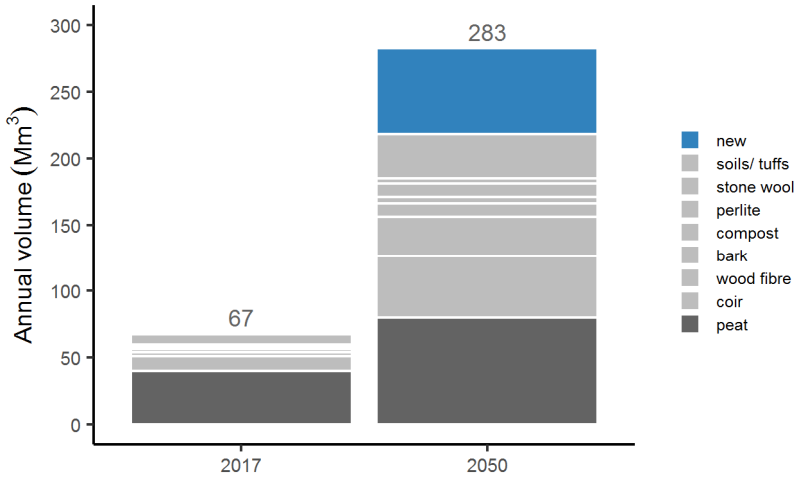

Figure 1.2 Estimated global demand for soilless substrates in 2050 (adapted from Blok et al., 2021)

3. Commonly used growing media

Both inorganic and organic materials can be used as soilless substrates. For high value crops (e.g., tomatoes, cucumbers and peppers), stone wool, a pre-shaped inorganic material, is commonly used due to its inert nature and ease of handling (Savvas & Gruda, 2018). However, the production process of new stone wool and the disposal of used substrates have negative impacts on environment, which has led to demands for renewable growing media to replace stone wool. Other inorganic materials such as perlite, pumice, vermiculite etc. are often added with a small proportion to improve certain physical properties of organic substrates.

Compared to inorganic materials, organic materials take up a larger portion of growing media due to their widespread availability, relatively low cost, acceptable performance, convenience in disposal and low environmental impacts (Savvas & Gruda, 2018). Although diverse organic materials have been investigated for being used as substrates, only few materials have become commercial substrates including peat, coir, bark, wood fiber and compost.

3.1 Peat

Peat is partially decomposed plant materials of sphagnums, other mosses and sedges which accumulate under acid, waterlogged conditions and in the absence of nutrients and microorganisms (Bunt, 1988). Peat used in horticulture is harvested from peatlands which mostly locate in northern Europe, Baltic area and Canada. Half of horticultural peat extraction is from Germany and Canada using block-cut or vacuum harvest method (International Peatland Society; Waddington et al., 2009). After drainage and vegetation clearance, peatlands are cut into blocks (peat sods) to different depths. The sods are then air-dried until moisture content of the sods reaches 40–50% of weight and further crushed and screened to different fractions. In vacuum harvest, a shallow layer of the surface of peatlands is milled and turned for air-dry until peat moisture reaches 50–60% of weight. Peat is collected by vacuum harvesters, then stockpiled for several months before being further processed in the factory. Peat types

vary according to their botanical composition, degree of decomposition and trophic status (Bunt, 1988). The degree of peat decomposition is described via the H1 to H10 von Post humification scale including weakly decomposed (H1–H3), medium decomposed (H4–H6) and strongly decomposed (H7–H10) (Bunt, 1988). Horticultural peat is mainly composed of sphagnum and classified as 'white peat' or 'light peat' (H1–H3), 'dark peat' (H4–H6) and 'black peat' (H7–H10).

Properties of peat substrates are affected by botanical composition of peat, degree of decomposition, extraction method and processing (Michel, 2010). In general, sphagnum peat has low bulk density (0.08–0.26 g cm^{-3}), high total porosity (80–95%, v/v) with high water-holding capacity and low-to-moderate aeration (Bunt, 1988; Carlile et al., 2019). Sphagnum peat has acidic pH (3.5–4.1), high cation exchange capacity (CEC) (110–250 meq 100 g^{-1}). Containing different lignin-type to vascular plants and phenolic antimicrobial substances, peat is less susceptive to decomposition, thus less shrinkage (Carlile et al., 2019). Peat is generally free of pathogen and weeds.

Main limitations in peat properties include low re-wetting capacity at lower moisture content, acidic pH and low aeration (Fonteno et al., 2013; Michel, 2010; Michel et al., 2013; Michel, 2015). Peat amendment with clay, wetting agent or other hydrophilic materials like coir or wood fiber improve wettability of peat substrates (Durand et al., 2021; Fields et al., 2014; Fonteno et al., 2021). Although the low pH of peat is in favor to grow acid-loving plants, liming is often required to raise pH of peat to 5.5–6.5 in the cultivation of other common greenhouse crops. To improve aeration, different particle size fractions of different peat types can be mixed, or peat can be amended with aggregates materials such as perlite, bark or wood chip aggregates (Owen et al., 2016).

Having the longest history as soilless substrate since 1950s, peat is the most widely used substrate in horticulture. The performance of peat is reliable and consistent in many commercial cultivation settings; this has challenged the adoption of any new materials as peat alternatives.

3.2 Coir

Coir is the by-product of the coconut (*Cocos nucifera*) processing industry, consisting of the dust and short fibers derived from the mesocarp of coconut fruits. Coir is primarily produced in Sri Lanka, India, Philippines, Indonesia, Mexico, Costa Rica and Guyana (Abad et al., 2002). Coconut husks are typically soaked in saline water, i.e. retting process, to soften, then grinded to extract the long fibers which are used for multiple industrial applications. The remaining dust and short fibers are further processed for horticultural uses. As the husks can accumulate salts via the saline soil in coconut plantation and/or via the retting process with saline water, coir materials are washed with fresh water to leach salts and/or further washed with buffer solution of calcium nitrate to enhance nutrient availability in substrate solution. After drying, coir materials will be dry-heated for short duration to kill potential weeds and pathogen (Menses & Borin, 2021). Compressed coir bales are then transported to substrate manufacturers in Europe and North America.

Coir has low bulk density (0.05–0.08 g cm^{-3}), high pore volume with excellent balance of water holding capacity and air volume (Abad et al., 2005; Fornes et al., 2003). Coir is hydrophilic material as it has no waxy cutin to repel water (Michel et al., 2017). Coir has stable and moderate pH 6 without lime requirement. CEC ranges from 39-60 meq 100 g^{-1} (Evans et al., 1996). Due to high lignin and less cellulose content, coir has low risk for nitrogen immobilization and less shrinkage (Bunt, 1988; Carlile et al., 2019).

Coming from different production sites which have different growing conditions for coconut palms, processing practices of the coconut husks and the age of stockpiled coir dust, large variance in substrate performance is an issue in coir (Abad et al., 2002). At the beginning of coir trials in the early 1990s, coir was reported to release of sodium (Na^+), chlorine (Cl^-) and potassium (K^+) and fix calcium (Ca^{2+}), thus reduced plant growth. As coir dust contains a certain amount of exchangeable K^+, Na^+, Ca^{2+} and Mg^{2+} on the surface of coir particles, Ca^{2+} and/or Mg^{2+} from fertilizers can be fixed into coir and K^+ and Na^+ from coir will be desorped causing nutrient imbalance in root-zone solution (Verhagen, 1999). Additional washing with water and buffering with calcium nitrate enhance nutrient availability in coir. However, these processing steps add additional cost into coir products. With transportation cost, coir is relatively more expensive than peat.

Since the first trials in 1990s, the performance of coir has been well understood and improved. Coir has increased its use in soilless culture as propagation substrate, potted substrate, growbag substrate and decomposable press pots for wide range of crops. It can also be used as stand-alone substrate, i.e. without mixing with other substrate components (Xiong et al., 2017). Although coir is considered as an excellent material to replace peat, its potential volume is limited as the supply capacity of coir dust depends on coconut processing industry and the competition from other industries for fiber use (Blok et al., 2021). Long transportation from Asia also causes environmental concerns and increases the cost (Barrett et al., 2016).

3.3 Bark

Bark, a by-product of the wood and paper industry, includes the inner bark (living phloem) and the outer bark (rhytidome) (Bunt, 1988). Bark is common substrate in areas where peat is scarce or expensive and lumber activities are predominant, such as in southern Europe, southern eastern US, Australia and New Zealand (Bunt, 1988; Carlile et al., 2019). Softwood bark from pine trees is predominantly used as substrate. In some areas in western US where pine bark is not available, hardwood bark from redwood is used instead (Bunt, 1988). After being debarked from the logs, large bark will be hammermilled, then aged or composted to reduce phytotoxicity and enhance nutrient availability before being used to grow plants.

Pine bark has slightly higher bulk density than peat (0.25–0.45 g cm^{-3}), lower total porosity (84%) with high air volume (33%) and moderate water holding capacity (51%) (Bunt, 1988; Carlile et al., 2019). Pine bark has acidic pH 4–5, CEC in the range of 38–98 meq 100 g^{-1} between pH 4 and 7 (Daniels

& Wright, 1988). Bark can have antagonistic effects and offer suppression of root diseases including *Phythium* and *Phytophthora* (Bunt, 1988).

Two major problems in bark substrate are N immobilization by either microorganism or physical fixation of nitrogen within the bark particles and the presence of phytotoxicity (Bunt, 1988). Both fresh and aged Douglas fir bark had high N immobilization (70–80% N immobilized after 4-day incubation with N solution of 75 mg N L^{-1}) (Buamscha et al., 2008). Bark contains phenols, tannins, manganese and other compounds which have phytotoxic effects on seed germination and plant growth. Composting or aging are the common practices to reduce phytotoxic substances and N immobilization in fresh bark.

As cost-effective material, from the 1960s onwards, bark has been increasingly used in horticulture. However, the changing lumber industry could limit the supply capacity of this material (Altland, 2010).

3.4 Wood fiber

Wood fiber is defined as the product of a process in which wood undergoes thermomechanical fraying and possibly treatment with conditioners for horticultural purposes (Schmilewski & Nordzieke, 2019). Fresh wood chips from debarked softwoods (conifers) are extruded with high pressure and high temperature. The process changes the structure of the material, volatilizes phytotoxic substances from the fresh wood and sterilizes the material. Wood fiber is then screened into different fractions for final products.

Properties of wood fiber substrates depend on wood types, the defibration methods and particle size fractions. In general, wood fiber substrates have similar bulk density (0.08–0.20 g cm^{-3}) and total pore volume (90%) as peat, with lower water retention and higher aeration (Gruda & Schnitzler, 2004). Wood fiber has high re-wetting capacity (Michel et al., 2017). Wood fiber has pH 3.8–6.6 with low CEC (2–15 meq 100 g^{-1}) and low pH buffering capacity (Carlile et al., 2019).

N immobilization and shrinkage are main issues in wood fiber substrates. To overcome these issues, additional N can be impregnated into wood fiber during the fraying process or additional N can be added via fertilization during plant cultivation (Gruda et al., 2000).

Wood fiber is an important soilless substrate component to enhance wettability and aeration of other growing media (Durand et al., 2021). It is expected to continue developing with sufficient supply capacity (Blok et al., 2021).

3.5 Composts

Compost is a general term describing all organic matter that has undergone a long, thermophilic, aerobic decomposition process, i.e. composting (Raviv, 2005). Composting is a sustainable practice to recycle the organic wastes and the final product as composts is mainly used as soil improver. To avoid cultivation risks in soilless culture, the use of composts as growing media requires stricter regulations on the feedstock, quality of the compost product and the proportion of composts in substrate mixes. Green composts or composted green wastes produced from plant materials are the most commonly used

compost as soilless substrate in Europe, mainly for the hobby market (Carlile et al., 2015). For professional growers, stricter requirements for feedstock is applied to ensure a safe end product. For example, the RHP certificate for green composts does not allow all kinds of green waste and green waste from agricultural and horticultural activities as they pose high risk of plant pathogen (Wever & Scholman, 2011). Also the maximum proportion of composts in the substrates should not excess 20% of volume.

Compost properties vary considerably depending on feedstock materials, composting methods and stability of composts. In general, the advantages of composts as growing media are nutritional contribution (Raviv, 2005) and suppressive against soil-borne diseases (Raviv, 2011).

Limitations on compost performance are high bulk density (0.25–0.55 g cm^{-3}) which increases transportation cost, low water holding capacity, high pH, high salinity, shrinkage and biological instability (Raviv, 2013). To overcome these limitations, several practices are recommended such as adding elemental sulfur to reduce compost pH (Costello & Sullivan, 2014), mixing composts with other substrate materials to reduce salinity, pH and enhance substrate stability (Raviv, 2013; Taylor et al., 2016). The main constraint on using composts as a professional substrate is the inconsistency of compost products between the batches and years. To ensure the quality of compost products, several standards for composts as growing media have been developed in Europe such as the RHP in the Netherlands, the RAL (Gütezeichen Kompost) compost standards in Germany and the PAS100 standards in the UK (Barrett et al., 2016).

Due to the potential large volume of waste feedstock and political pressure to reduce landfill waste, as low cost material, supply capacity of composts is prospected to increase rapidly, mainly for the hobby market. The application of composts as a professional substrate takes smaller share.

4. Promising new substrates: valuable waste streams and renewable primary materials

A wide range of materials have been investigated as soilless substrates, which belongs to two main categories as valuable waste materials and renewable primary materials (Barrett et al., 2016). The first group has been studied to a greater extent due to the urgent need to recycle and/or valorize the large volume of various types of waste. The main concerns for this material are the consistent performance of the substrates and the long-term security of supply (Barrett et al., 2016). For example, biochar, a pyrolysis byproduct from various waste feedstock, could be promising as substrate component (De Tender et al., 2016). However, the consistency of biochar performance still need further investigation. Pine tree substrate is produced from the whole pine tree via minimal processing methods such as milling and screening without aging or composting. With proper substrate processing and additional nitrogen supply, pine tree substrates show reliable and consistent performance in soilless culture (Jackson et al., 2008, 2010). This substrate is very promising in replacing peat and pine bark in the southern US where the forestry activities are much common (Jackson & Wright, 2009). As the wood feedstock depends on the forestry industry, long-term security of the supply is an important concern (Barrett et al., 2016).

Compared to the waste materials, an advantage of renewable primary materials is the better predictability of the quantity and the chance to manage quality of the feedstock. The renewable primary materials have been studied to a lesser extent (Barrett et al., 2016), mainly due to the availability of the materials on-site. To develop a renewable primary material into a commercial substrate, besides the substrate performance, the main challenges are the potential supply capacity of the feedstock and the competition for feedstock from other industrial applications. Substrate manufacturers have to either establish their own production sites to grow the primary biomass or purchasing the biomass from the farmers. Therefore, a promising primary material should be regionally available (reduce transportation cost), low-input crop with potential high yield (reduce investment into the biomass production) and possible less competition for the primary feedstock from other industrial application (to reduce market competition).

Sphagnum biomass and biomass from bioenergy crops are the two primary materials which have been investigated (Barrett et al., 2016; Schmilewski & Köbbing, 2016). Sphagnum biomass cultivated on degraded or drained peatlands is a suitable soilless substrate; however, the biomass accumulation of sphagnum is very low, e.g. about 0.8 kg m^{-2} dry biomass after seven growth seasons (Pouliot et al., 2015). Bioenergy-based substrates, derived from bioenergy crops such as miscanthus (*Miscanthus* x *giganteus*), switchgrass (*Panicum virgatum*), willow (*Salix* spp.) and reed (*Phragmites australis*), could be used as substrate constituents to grow crop in soilless culture (Altland, 2010; Altland & Krause, 2009; Cárthaigh et al., 1997; Kraska et al., 2018; Kraska & Pude, 2019; Kuisma et al., 2014; Vandecasteele et al., 2018). The potential supply capacity of bioenergy-based substrates is large, as bioenergy crops are fast-growing crops with low input requirements. However, substrate performance need to be further investigated for better nutrient availability.

5. Miscanthus, a renewable primary substrate: potentials and challenges

Among the bioenergy-based substrates, miscanthus is one of the most prominent material due to its highest biomass yield in the temperate regions with low environmental impacts (low-input crop, 10–25 t dry matter ha^{-1} annually, carbon sequestration and ecosystem services) (Clifton-Brown et al., 2004; Emmerling & Pude, 2017; Heaton et al., 2010; Lewandowski et al., 2000). Results showed that miscanthus straw could be used as substrate constituent in container mixes (20-80% of volume) for nursery shrubs (Altland, 2010; Bąbelewski et al., 2019; Cárthaigh et al., 1997; Frangi et al., 2012; Pancerz & Bąbelewski, 2019), tomatoes (Guo-jing et al., 2002), wood cuttings (Bąbelewski & Pancerz, 2018), strawberries (Debode et al., 2018; Kraska & Pude, 2019) and as stand-alone substrate, i.e. sole constituent, in growbag for tomatoes and cucumbers (Kraska et al., 2018). While stand-alone miscanthus in growbags showed comparable plant yield to control substrate (stone wool) (Kraska et al., 2018), increasing proportion of miscanthus in container mixes led to decreasing plant growth (Frangi et al., 2012). The advantages of the substrate are enhancing aeration in the mix with peat (Altland & Locke, 2011) and carrying biological control agent *Trichoderma* spp. (Debode et al., 2018). Under circular

economy, as bioenergy material, used miscanthus substrates could be further used for direct combustion in a cascading manner (Kraska et al., 2018).

The challenges in substrate performance are low water holding, slightly alkaline pH and N immobilization, thus biological instability (i.e. substrate shrinkage). Although the substrate has been first investigated since late 1990s, miscanthus has still not been a commercial substrate. *Two main challenges* are (i) evaluating the potential supply capacity of miscanthus feedstock, opportunities and challenges when developing this primary material; (ii) optimizing substrate performance such as increasing water holding capacity, manipulating substrate pH in the optimal range for greenhouse crops and reducing nitrogen immobilization. While solving the first challenge would provide fundamental information on the feasibility of developing miscanthus into commercial substrate, the second would increase the proportion of miscanthus use in the substrates.

6. Thesis outlines

The general objectives of this PhD work

- to assess the quality of miscanthus as primary feedstock and the performance of miscanthus substrate, to estimate potential supply capacity of miscanthus feedstock and to analyze strengths, weaknesses, opportunities and threats (SWOT) when developing miscanthus into commercial soilless substrate by conducting a literature review
- to optimize the performance of miscanthus substrate by conducting trials using the 'stand-alone substrate approach'

Chapter 2 "Evaluation of *Miscanthus* as renewable primary feedstock for the soilless substrate market – A review"

The aim was to evaluate the feasibility of developing miscanthus into commercial substrate.

The objectives were (i) to briefly evaluate miscanthus as feedstock including production aspects of miscanthus crop and properties of the feedstock which might determine properties of miscanthus substrate, (ii) to assess the performance of miscanthus soilless substrate, (iii) to estimate the potential supply capacity of miscanthus feedstock regarding land availability, potential yield and competition from other industries and (iv) finally to conduct a strengths, weaknesses, opportunities and threats (SWOT) analysis for developing miscanthus into a commercial substrate.

Chapter 3 "Miscanthus as stand-alone substrate in growbags for tomatoes with cascade utilization as direct combustion"

The aim is to introduce miscanthus as stand-alone substrate to grow tomatoes in growbags with an additional benefit as direct combustion of the used substrates.

The objectives: The growth of tomato plants in growbag substrates were investigated in two experiments in 2018 and 2019. The experiment in 2018 tested the effects of miscanthus genotypes as substrates under

specific pH and nutrient strength of input solution on (i) pH and nutrient strength in root-zone solution, (ii) on plant performance and (iii) on combustion quality of used substrates. The experiment in 2019 tested the effects of substrate particle size and substrate amendments with elemental sulfur or $Ca(NO_3)_2$ on (i) pH and nutrient strength in root-zone solution and (ii) on plant performance. The tomato growth in 2018 and 2019 was used to evaluate the consistent performance of miscanthus substrates.

Chapter 4 "Primary mechanical modification to improve performance of miscanthus as stand-alone growing media"

The aims are (i) to understand the substrate performance of miscanthus as stand-alone substrate and (ii) to improve the performance via mechanical modification

The objective is to investigate how different mechanical processing methods influence miscanthus substrate morphology, hydrological properties, pH and N immobilization.

Chapter 5 "Effects of washing and steaming pretreatments on reducing nitrogen immobilization in miscanthus substrates"

The aim is to improve the performance of miscanthus substrate via secondary modifications

The objective is to assess the effects of steaming and washing miscanthus substrate on reducing nitrogen immobilization in miscanthus substrates.

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Chapter 2

Evaluation of *Miscanthus* as renewable primary feedstock for the soilless substrate market–A review

Highlights

- As renewable primary material, miscanthus substrate has advantage in quantity and quality control.
- Miscanthus could be used as substrate constituent in pots and as stand-alone substrate in growbags providing comparable plant growth to standard substrates.
- Potential supply capacity of the feedstock was estimated to be large.
- Strengths, weaknesses, opportunities and threats of developing miscanthus into a commercial substrate were analyzed.

Abstract

To meet the increasing demand for soilless substrates in the context of limited availability of peat and other key substrate components, substrate manufacturers are searching for new alternative raw materials. Compared to the waste stream materials, an advantage of renewable primary materials is the better predictability of the quantity and the chance to manage quality of the feedstock. Dry biomass of *Miscanthus* spp. is a promising primary material in the temperate regions because of its high biomass production under low-input and ecosystem services. Despite the studies have shown potential use of miscanthus as soilless substrate, miscanthus has not yet been adopted into the substrate market. This review evaluates the feasibility of developing miscanthus into a commercial substrate. Two main impediments to the adoption are (1) the challenges in miscanthus performance as low water holding capacity, high pH and risk of nitrogen immobilization which limit until now the proportion of miscanthus in the substrates, (2) the limited availability of miscanthus materials on-site due to the still small market for miscanthus in Europe. To develop miscanthus into commercial substrate, further studies should focus on modifications concerning cultivation management and substrate properties. Once reliable and consistent performance of miscanthus could be established under commercial growing conditions, it will create market for miscanthus as feedstock for soilless substrate. Given potential large land availability for growing miscanthus and relatively small market for miscanthus in Europe, competition for the feedstock seems not to be intense at present, but uncertain in the future. Cascade utilization of used miscanthus substrates would not only increase resource efficiency and by that sustainability, but also add additional market opportunities to reduce competition for the feedstock.

Key words: Peat alternatives, soilless substrate, soilless culture, supply capacity, SWOT analysis

Abbreviations: AFP: air-filled porosity, EC: salinity conductivity, SWOT: strengths, weaknesses, opportunities and threats, WHC: water holding capacity.

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1. Introduction

Soilless culture is the cultivation of plants in systems without soil in situ (Gruda, 2009), in which plant roots mainly develop in porous materials named 'growing media' or 'substrates'. As soil replacement, soilless substrate is a key factor in the success of crop production. Several materials which have been extensively used as substrate constituents include peat, coir, pine bark, wood fiber and composts (as organic materials) and stone wool, perlite (as inorganic substrates) (Barrett et al., 2016; Schmilewski, 2017).

Based on the increasing demand for food and ornamental crops from both professional and hobby market, worldwide demand for growing media in 2050 is projected to exceed its recent volume by four times (Blok et al., 2021). Taking into account the potential supply capacity of key substrates, to meet the demand, substrate industry will need approximately 65 Mm^3 of new alternative growing media annual globally (Blok et al., 2021). Together with promoting future propagation techniques which would employ less volume of substrates or no substrate at all, searching for new alternative raw materials is a key measure of substrate manufacturers (Röse, 2019; Schmilewski, 2012).

Promising alternative substrates should have reliable and consistent performance, large supply with affordability and low environmental impacts (Barrett et al., 2016). A wide range of new raw materials has been investigated comprising of waste or by-product materials from the industry and renewable primary sources (Barrett et al., 2016; Gruda, 2019). While the waste stream materials have been extensively investigated due to the availability of the materials and the urgent need to valorize them, the challenges in developing these materials into commercial substrates are inconsistent quality of the feedstock, additional processing cost and uncertain long-term supply (Barrett et al., 2016).

The renewable primary materials have been studied to a lesser extent (Barrett et al., 2016), mainly due to the availability of the materials on-site. Compared to the waste materials, an advantage of renewable primary materials is the better predictability of the quantity and the chance to manage quality of the feedstock. To develop a renewable primary material into a commercial substrate, the main challenges are the potential supply capacity of the feedstock and the competition for feedstock from other industrial applications. Substrate manufacturers have to either grow primary biomass by themselves or purchase the feedstock from the farmers. Therefore, a promising primary material should be regionally available (to reduce transportation cost), low-input crop with potential high yield (to reduce investment into the biomass production) and possible less competition for the primary feedstock from other users (to reduce the selling price of feedstock).

Plant fibers from bioenergy crops have been investigated as soilless substrate since the late 1990s. The materials include dry biomass of miscanthus (*M.* x *giganteus*) (Altland, 2010; Cárthaigh et al., 1997; Guo-jing et al., 2002; Jensen et al., 2001a; Kraska et al., 2018; Vandecasteele, Viaene, et al., 2017), switchgrass (*Panicum virgatum*) (Altland & Krause, 2009), willow (*Salix spp*.) (Altland, 2010) and reed (*Phragmites australis*) (Frangi et al., 2012; Kuisma et al., 2014; Vandecasteele et al., 2018).

Among the bioenergy-based substrates, miscanthus represents one of the most prominent material in the temperate regions due to its highest biomass yield (10–25 t dry matter ha^{-1} annually) under low-input demand and ecosystem services (soil carbon storage, biodiversity and carbon dioxide mitigation) (Clifton-Brown et al., 2004; Emmerling & Pude, 2017; Heaton et al., 2010; Lewandowski et al., 2000). Results showed that miscanthus straw could be used as substrate constituent in the container substrates (20-80% of volume) for nursery shrubs and bedding plants (Altland, 2010; Bąbelewski et al., 2019; Cárthaigh et al., 1997; Frangi et al., 2012; Jensen et al., 2001a; Pancerz & Bąbelewski, 2019), tomatoes (Guo-jing et al., 2002), wood cuttings (Bąbelewski & Pancerz, 2018), strawberries (Debode et al., 2018; Kraska & Pude, 2019) and as stand-alone substrate, i.e. sole constituent, in growbags for tomatoes and cucumbers (Kraska et al., 2018; Nguyen et al., 2021).

Despite being investigated as soilless substrate since the late 1990s, miscanthus has not yet been a commercial substrate. The remaining question is what factors might impede the adoption of miscanthus in the substrate market. To determine major impediments and evaluate the feasibility of developing miscanthus into a commercial substrate, this review (i) briefly evaluates miscanthus as feedstock including production aspects of miscanthus crop and properties of the feedstock which might determine properties of miscanthus substrate, (ii) assesses the performance of miscanthus substrate in soilless culture, (iii) estimates the potential supply capacity of miscanthus feedstock regarding land availability, potential yield and competition from other industries and (iv) finally conducts a strengths, weaknesses, opportunities and threats (SWOT) analysis for developing miscanthus into a commercial substrate.

2. Miscanthus as the feedstock

2.1 Background on miscanthus crop production

Miscanthus, a perennial rhizomatous C4 grass, originates in the tropics and subtropics (Greef & Deuter, 1993). It was first introduced to Denmark in 1935 from Japan, then was propagated and distributed to other European countries in the late 1970s as ornamental plants. The yield trials started in 1983 and then developed quickly in the early 1990s for the bioenergy purpose (Clifton-Brown et al., 2015). Due to its high cellulose content and remarkable adaptability to different environments, miscanthus has been cultivated widely in the temperate regions (Europe and North America) mainly for solid fuel and other bio-based sectors such as building and packaging materials, animal bedding and chemical productions (Ben Fradj et al., 2020; Lewandowski et al., 2000).

Miscanthus x *giganteus*, a natural hybrid from *M. sacchariflorus* and *M. sinensis* (Greef & Deuter, 1993; Hodkinson et al., 2002), is the only commercial genotype (Jørgensen, 2011). Several synonyms which have been used for *M.* x *giganteus* include *M. sinensis* var. 'Giganteus', *M.* x *ogiformis* Honda 'Giganteus' and *Miscanthus* 'Giganteus' (Lewandowski et al., 2000). As a sterile triploid, *M.* x *giganteus* cannot produce seeds. To establish miscanthus stand, rhizomes or tissue culture plantlets have been used as propagation materials. Besides *M.* x *giganteus*, the species *M. sinensis* and *M.*

sacchariflorus have been studied the most, but only in the experimental scale (Arnoult & Brancourt-Hulmel, 2015).

The plantation starts in spring time. Planting materials are rhizomes, tissue culture plantlets and seeds (Lewandowski et al., 2000). Planting with rhizomes is the most common practice due to the high cost of tissue plantlets and the low establishment of seeds (Xue et al., 2015). During vegetative growth, miscanthus plants can reach a height up to 4 m. Plant senescence occurs in autumn resulted in leaf fall, dry out biomass and nutrient relocation from above-ground biomass into underground rhizomes. Harvest can be done in late winter before the emergence of new sprouts in early spring. At the harvest, moisture content of materials is about 15% of weight so the harvests can be stored without additional drying. After the first year of establishment, the dry biomass can be harvested from the second year onwards. In the establishment phase (i.e. the 3^{th} to 4^{th} year of cultivation), the yield is rather low. After the establishment phase, miscanthus stand enters high biomass production phase with the average annual yield could reach 20 t dry matter ha^{-1} (Lewandowski et al., 2000). The yield difference depends on location and soil types with a reported yield range of 5–55 t dry matter ha^{-1} (Heaton et al., 2010). Fertilization is required only during establishment phase (60 kg N ha^{-1}) (Lewandowski et al., 2000), after that no fertilizer requirement as the nutrients and carbohydrates stored in the rhizome during winter will be mobilized into shoots in spring. Biological nitrogen fixation also contributes to nitrogen use efficiency in miscanthus (Keymer & Kent, 2014; Liu & Ludewig, 2019). Weed management is very crucial during the first two years of establishment phase. After the stand has established, weed management is no longer needed. A miscanthus stand could be extended up to 15-20 years.

2.2 Properties of miscanthus as feedstock for soilless substrate

The low moisture content of harvested miscanthus is advantageous in transportation and storage of the substrate. Harvested materials include mainly stem biomass with small portion of remaining leaf biomass. Cross section of stem internode separates the inner porous parenchyma pith with the outer stem; from outer stem towards pith center, the pore density decreases but pore size increases (Botto et al., 2014; Klímek et al., 2018; Pude et al., 2004). The porous parenchyma pith creates inner pore system for water and air. The leaf biomass and outer stem containing natural waxes in the cuticle (Attard et al., 2016), which could affect the wettability of miscanthus substrate.

As plant fibers, miscanthus is rich in cellulose, hemicellulose and lignin. Compared to other plant fibers as soilless substrates such as peat, coir and wood fiber, miscanthus has higher cellulose, similar adequate hemicellulose and lower lignin content (Table 2.1). This indicates potential different decomposition rate of the substrates. In miscanthus stem, lignin and cellulose are mainly in the cells around the vascular bundles with decreasing concentration from outer stem ring towards the pith (Kaack et al., 2003; Pude et al., 2004). High silicon (Si) content in miscanthus biomass could be potential as biogenic Si fertilizer (Houben et al., 2014; Monti et al., 2008) enhancing plant strengthening to against biotic and abiotic stress (Guntzer et al., 2012).

Miscanthus harvest is abundant with endophytic bacteria and fungi (Schmidt et al., 2018). Together with the carbon (C) content from the material, miscanthus substrate poses risk for N immobilization. The endophytic microorganism, however, could have antagonism effect on plant pathogens.

Table 2.1 Plant fiber composition of miscanthus, peat, coir and wood fiber

Substrate	Plant fiber composition (%, dry weight)		
	Cellulose	Hemicellulose	Lignin
Miscanthus [z]	46-69	20-30	11-14
Peat [y]	17	38	26
Coir [y]	32	43	45
Wood fibre [x]	47-49	18-26	27-30

[z] (Hodgson et al., 2011; Pude et al., 2005; Vandecasteele et al., 2018)
[y] (Abad et al., 2002)
[x] (Domeño et al., 2011; Guo-jing et al., 2002)

3. Performance of miscanthus as soilless substrate

3.1 Literature search for studies on miscanthus as soilless substrate

Literature on miscanthus as soilless substrate was searched in following scientific databases including Web of Science, Science Direct and Google Scholar using the following search syntax as "miscanthus AND ("growing media" OR "growing medium" OR "growing substrate" OR soilless OR compost*)" by the date 10 June 2021. After screening the titles and abstracts to exclude irrelevant publications, 13 publications on composted miscanthus and 15 publications on non-composted miscanthus are included (Table 2.2).

Table 2.2 List of publications on miscanthus (*Miscanthus* spp.) as soilless substrate

Substrate processing	Objectives of the studies	Substrate treatments	Tested crop	References
Composted	Monitor C/N turnover, lignocellulose degradation and microbial community change during composting process Monitor physical properties and structural changes during composting process	Shredded miscanthus straw (60 x 5 x 2 mm, length x width x thickness) mixed with N sources including pig slurry, urea, $NH_4(NO_3)$ at varying composting lengths (3–12 months)	na	(Eiland, Klamer, et al., 2001; Eiland, Leth, et al., 2001; Klamer et al., 2001; Klamer & Bååth, 1998; Klamer & Søchting, 1998; Leth et al., 2001) (Clemmensen, 2004; Dresbøll, 2008; Dresbøll & Magid, 2006; Dresbøll & Thorup-Kristensen, 2005)
	Assess plant growth on composted miscanthus (nursery shrubs)		*Hedera helix*, *Fatsia japonica*	(Jensen et al., 2001a, 2001b, 2002)
Non-composted	Assess the performance of miscanthus substrates as container substrate for nursery shrubs and bedding plants	Chopped or fibrous (extruded) miscanthus mixed with sphagnum peat with varying ratios of 40:60, 50:50, 70:30, 100:0	*Hypercium patulum*, *Ligustrum vulgare*	(Cárthaigh et al., 1997)
		Hammermilled miscanthus (screen size of 0.95 cm) mixed with municipal solid waste compost and sphagnum peat with the ratio of 70:10:20	Annual vinca	(Altland, 2010)
		15% sphagnum peat, 5% municipal solid waste compost mixed with varying ratios of pine bark and hammermilled miscanthus (screen size of 0.48 cm)	Hibiscus	(Altland & Locke, 2011)
		Extruded of field-chips miscanthus mixed with peat and pumice with varying ratios of 25:60:15, 50:40:10, 75:20:5	*Prunus laurocerasus* *Viburnum tinus*	(Frangi et al., 2012)
		Miscanthus mixed with peat with varying ratios of 100:0, 70:30, 50:50, 30:70, 0:100, amended with hydrogel and two levels of fertilizer treatment	*Hydrangea arborescens*	(Pancerz & Bąbelewski, 2019)
	Assess the performance of miscanthus substrates as rooting substrate for nursery shrubs	Miscanthus mixed with peat and sand with varying ratios of 30:65:5, 50:45:5, 70:25:5, 95:0:5	*Spiraea*, *Taxus*, *Cotoneaster*, *Euonymus*	(Bąbelewski & Pancerz, 2018)
	Assess the performance of miscanthus substrates as container substrate for soft-fruits	Field-chips miscanthus mixed with fertilized commercial substrate (20:80, v) amended with additional fertilizer	Strawberries	(Vandecasteele, Viaene, et al., 2017)
		Field-chips miscanthus or extruded miscanthus or extruded miscanthus pre-	Strawberries	(Debode et al., 2018)

Substrate processing	Objectives of the studies	Substrate treatments	Tested crop	References
		colonized by *Trichoderma,* mixed with a fertilized commercial growing medium for strawberry with a ratio of 20:80		
		100% field-chips miscanthus (control, aged), miscanthus mixed with green compost (75:25, 50:50), miscanthus mixed with spent mushroom substrate compost (75:25, 50:50)	Strawberries	(Kraska & Pude, 2019)
	Assess the performance of miscanthus substrates as growbag substrate for tomatoes and cucumbers	Field-chips miscanthus mixed with wood fibers (80:20)	Tomatoes	(Guo-jing et al., 2002)
		Hammermilled, mechanical-frayed and extruded miscanthus substrates, stand-alone substrate (100% miscanthus)	Tomatoes, cucumbers	(Kraska et al., 2018)
		Hammermilled miscanthus from three genotypes: *M.* x *giganteus*, *M. sinensis* and *M. sacchariflorus* var. Robustus	Tomatoes	(Nguyen et al., 2021)
	Characterize substrate parameters without plant growth test	Field-chips miscanthus	na	(Vandecasteele, Dias, et al., 2017)
	Assess the effects of defibration, acidification or inoculation with biocontrol fungi on N immobilization	Defibration: miscanthus processed with extrusion, retruder, disc refining or steam explosion. Acidification: sterilized extruded miscanthus straw mixed with citric acid or acetic acid Inoculation with biocontrol fungi: sterilized extruded miscanthus straw inoculated with *Trichoderma*, *Metarhizium*, *Chaetomium*, *Gliocladium*	na	(Vandecasteele et al., 2018)
	Monitor the dynamic change of C, N in miscanthus substrates during plant cultivation (no report on plant growth)	Miscanthus mixed with peat with varying ratios of 100:0, 70:30, 50:50, 30:70, 0:100, amended with three levels of fertilizer treatment	*Spiraea japonica* (no report on plant growth)	(Bąbelewski et al., 2019)

na: non-applicable. Field-chips: harvested miscanthus from the field using forage harvester (maize harvester). The studies used *M.* x *giganteus* as the feedstock, except where specific *Miscanthus* species mentioned.

3.2 Rationales for selecting miscanthus as the feedstock for soilless substrate

The first reported studies on miscanthus as soilless substrate were from Germany and Denmark (Cárthaigh et al., 1997; Klamer & Bååth, 1998; Klamer & Søchting, 1998) in the late 1990s. A reason for selecting miscanthus as feedstock is the availability of miscanthus on-site. During that time, the field trials of miscanthus developed first in Germany and Denmark which resulted in the availability of miscanthus for substrate experiments. Other rationales for selecting miscanthus are defined material feedstock for composting (Klamer & Bååth, 1998), local cost-effective material (Altland, 2010) and sustainable feedstock (Guo-jing et al., 2002; Kraska et al., 2018; Vandecasteele, Viaene, et al., 2017).

3.3 Miscanthus as composts

Studies on composted miscanthus assessed the effects of N sources and composting age on the physical, chemical and microbial changes during composting process, properties of compost end product as growing substrate and plant growth. Shredded miscanthus with size from 2–5 cm was used as C source. Tested N source included ammonium sulfate, urea and pig slurry. Composting process lasted from 8 weeks to 15 months.

3.3.1 Changes during composting process

In general, temperature change inside miscanthus compost pile occurred in three phases including before heat peak, at heat peak and after heat peak. After mixing, compost temperature increased up to 50^{o}C, reached its maximum temperature around 60–70^{o}C (at heat peak) and then reduced gradually to ambient value of around 25^{o}C. Subsequent turning and rewetting compost piles reheated the compost. Composts with different N sources reached their maximum temperature at different rates and values. For example, miscanthus composted with urea, ammonium sulfate and pig slurry reached their heat peak at 41, 60 and 65^{o}C after three, two and one days (Eiland, Klamer, et al., 2001; Eiland, Leth, et al., 2001; Klamer & Bååth, 1998). After the heat peak, compost temperature reduced gradually to 40^{o}C around day 10 or day 20, then reached 25^{o}C over months of composting. Regarding to temperature change, miscanthus composted with pig slurry indicated the most efficient decomposition as the compost reached highest temperature peak within shortest time.

The initial C/N ratio of miscanthus compost affected decomposition rate and microbial community of compost (Eiland, Klamer, et al., 2001). Miscanthus straw was mixed with pig slurry solutions at different concentrations from 100%, 30%, 10%, 3% to 0% of weight resulted in different initial C/N ratios of 11, 35, 47, 50 and 54, respectively. While compost treatment with the lowest C/N ratio of 11 did not change its value, other treatments with higher C/N ratio reduced their values rapidly after three months, then remained stable until the end of composting. Nitrate release was faster and at higher amount in miscanthus composted with higher concentration of pig slurry (initial C/N of 11 to 47). Compost treatments of lower C/N ratio decomposed fiber faster than those of higher C/N ratio. Hemicellulose degraded to the largest extent, with the amount of hemicellulose degraded after 12

months of composting of nearly 100% and 70% at lower and higher C/N ratio, respectively. Cellulose degraded to a lower extent, with more than 60% of cellulose degraded in compost with lowest C/N ratio and 35–60% degraded in composts with higher C/N ratio. Lignin did not decompose during composting. Initial C/N ratio of compost also showed different microbial biomass and community. At the early stage of composting (within the first month), microbial biomass in composts with low C/N ratio was higher than that with higher C/N. After 12 months the trend was opposite. While the compost with lowest C/N ratio had bacteria-dominated community, the composts with higher C/N ratios increased the fungal biomass.

Microorganism biomass and community change during composting process was related to change in compost temperature (Klamer et al., 2001; Klamer & Bååth, 1998; Klamer & Søchting, 1998). Total microbial biomass increased to its maximum value at heat peak (six times higher than initial biomass), then reduced gradually to a half during period after heat peak. In the first phase, mesophilic and thermophilic microorganism metabolized readily degradable compounds resulted in rapid increase in microbial biomass. Gram-positive bacteria increased rapidly with increasing temperature (a doubling time of approximately 4 hours during the first day of composting) and decreased with decreasing temperature. Gram-negative bacteria and fungi increased up to 50^{o}C, then decreased during heat peak. Above 60^{o}C, fungi became inactive and decomposition was mainly done by bacteria. Fungi started to increase again after the heat peak. Based on plating techniques, Klamer and Søchting (1998) identified dominating mesophilic and thermophilic fungi species occurred in miscanthus compost. Before the heat peak, dominating mesophilic species included *Penicillium* spp., *Absidia* spp., *Trichurus spiralis*, *Trichoderma* spp. After the heat peak, dominating thermophilic species included *Theromomyces lanuginosus*, *Scytalidium thermophilum* and *Peacilomyces variotii* (from day 15–27) and *Trichurus spiralis*, *Pesudallescheria boydii*, *Acremonium* spp. (from day 50–225). Around day 15 to day 50, a great number of fruiting bodies of *Coprinus cinereus* developed. The authors noticed that the appearance of *Asperigillus fumigatus* and *Pseudallescharis boydi* in compost might not cause a special health risk as these species distributed widely in the nature. *Trichoderma* spp., as biocontrol agent, also appeared in miscanthus compost at the initial phase, then reduced and increased again at the late stage of composting.

3.3.2 Quality of compost end product as soilless substrate

Type of nitrogen source determined physical properties of miscanthus compost (Clemmensen, 2004). Composted miscanthus with pig slurry or urea for 7 months showed similar physical properties to wood fiber and different to peat to some extent, whereas composted miscanthus with ammonium sulfate had different values. Bulk density of composted miscanthus with ammonium sulfate was the lowest (0.04 g cm^{-3}). Bulk density of composted miscanthus with pig slurry or urea (0.08–0.09 g cm^{-3}) was similar to wood fiber and two types of peat and lower than the other tested peat substrates. Porosity values were quite similar among tested growing media (93–96%). Air-filled porosity (AFP) of

miscanthus composted with pig slurry or urea was similar to wood fiber (AFP 60–65%) and much higher than all tested peat substrates (AFP 14–25%). AFP of miscanthus composted with ammonium sulfate was the highest (71–77%). The same trend was also observed for oxygen diffusion rate. Easily available water of substrates followed this order: miscanthus composted with urea, pig manure or ammonium sulfate (9–16%) < wood fiber (21%) < peats (34–43%).

The order of substrate pH was miscanthus composted with ammonium sulfate (pH 4.0) < miscanthus composted with urea (pH 5.9) ~ peats (pH 5.9) < composted with pig manure (pH 6.3) < wood fiber (pH 6.9). The lowest pH in miscanthus composted with ammonium sulfate might decrease the decomposition, thus resulted in high bulk density (Clemmensen, 2004). When composted with pig slurry, the concentration of pig slurry should not greater than 30% because of high electrical conductivity (EC) of compost product (Jensen et al., 2002). In general, miscanthus composted with pig slurry (concentration of 10%) or urea showed better substrate properties than miscanthus composted with ammonium sulfate.

Dresbøll et al. (2005, 2006, 2008) described the structural changes of substrate particles during composting of miscanthus with clover-grass as N source for eight weeks. Due to the lignification arrangement (throughout the stem), the decomposition of miscanthus was less obviously compared to that of hemp or wheat. Under the scanning electronic microscope, microbes showed only in the surface of miscanthus particles and were rarely found inside particles.

3.3.3 Plant growth on composted miscanthus

The performance of miscanthus composted with different N sources and at varying composting age was assessed in the cultivation of common ivy (*Hedera helix*) and Japanese aralia (*Fatsia japonica*) (Jensen et al., 2001a, 2001b, 2002).

In common ivy cultivation, miscanthus composted with pig slurry performed the best compared to miscanthus composted with other N sources (urea and ammonium sulfate). After 2.5 months of cultivation, rooted cuttings of common ivy grown in miscanthus composted with pig slurry (concentration of 10–30%, w/w) produced similar shoot length and shoot dry biomass to those in unfertilized and fertilized peats (Jensen et al., 2002). Increasing composting age from 3 to 12 months did not change plant growth, but increased water retention of compost from 40% to 70–80% (Jensen et al., 2002). Before being used in composting, pig slurry should be diluted to the concentration of 10–30% (w/w), lower or higher concentration caused decrease in plant growth (Jensen et al., 2001a, 2002). As observed in rooted cuttings of Japanese aralia, lowest shoot biomass was produced in miscanthus composted with pig slurry (Jensen et al., 2001a). The reasons could be the sensitivity of Japanese aralia to high salinity and excess levels of nutrients (K, P, Na, Cl, Mn, Cu and Zn) of miscanthus composted with pig slurry.

Although urea or ammonium sulfate might be safer N sources than pig slurry, miscanthus composted with one of these two N source for 7 months could not perform as well as fertilized and unfertilized

peats (Jensen et al., 2001b). Shrinkage of miscanthus composts occurred at greater extent than that of peats. (Jensen et al., 2001b). After 17 months of cultivation of common ivy, compost with ammonium sulfate or urea remained 77 and 84% of their initial volume, respectively. No moss, algae or weed growth was observed (Jensen et al., 2001a).

3.4 Miscanthus as non-composted substrates

After being harvested from the field, miscanthus straw can be further processed with a wide range of machinery resulted in different physical structures. The authors used different terms to describe their processing techniques. To make it clear, the following terms were used in this review to describe the processing methods: (i) field-chips for materials harvested from the field with forage harvester (or maize chopper); (ii) extruded/retruded (or fibers) for miscanthus processed with extruder or retruder; (iii) hammermilled (or chips) for miscanthus processed with a hammermill with a notice on screen size; (iv) shreds for materials processed with a mechanical fraying facility. For the materials which are not mentioned in (i)-(iv), the terms were used as those the authors reported with machinery name when applied.

3.4.1 Properties of miscanthus substrates

Miscanthus substrates had low water content (10-15%, w/w) with a bulk density in a range of 0.08–0.16 g cm^{-3} (Kraska et al., 2018). The dryness and lightness of miscanthus substrates was advantageous in transportation and indoor storage, but the substrate was dusty and prone to blowing when preparing substrate outdoor (Altland & Locke, 2011).

Particle size distribution of hammermilled miscanthus (with screen size of 0.95 cm) comprised 20% of fine particles (i.e. particle size smaller than 0.5 mm), 60% of medium particles (0.5–2 mm) and 20% of coarse particles (larger than 2mm) (Altland, 2010). Using smaller screen size of 0.48 cm did not change the proportion of medium particles (61%), but increase the amount of fine particles (37%) and reduce the fraction of coarse particles (2%) (Altland & Locke, 2011). Compared to pine bark processed in a same way, miscanthus substrate had similar proportion of fine particles (37% vs 25%), higher proportion of medium particles (61% vs 34%) and much lower quantity of coarse particles (3% vs 40%) (Altland & Locke, 2011).

Miscanthus field-chips had moisture properties similar to wood fibers, but lower than coir (Guo-jing et al., 2002). In detail, miscanthus and wood fibers had easily available water of 13%, water buffering capacity of 3% and air volume of 57%. Miscanthus can absorb water about three times of its own weight, while wood fibers and coir can absorb approximately seven and nine times, respectively. The water desorption and absorption curves showed that miscanthus had lower hysteresis than coir.

As the proportion of extruded miscanthus in the peat-miscanthus-pumice mixes increased from 25% to 75%, total porosity remained (90%), AFP increased from 26% to 40% and available water reduced from 30% to 23% (Frangi et al., 2012). A substrate mix of hammermilled miscanthus (screen size of

0.95 cm) with sphagnum peat moss and municipal solid waste compost (70:20:10, v) showed high AFP of 58% and low water holding capacity (WHC) of 34% (Altland, 2010).

Initial pH values of fresh field-chips and hammermilled miscanthus were in the range of 6.0–8.0 (Guo-jing et al., 2002; Nguyen et al., 2021; Vandecasteele et al., 2018). This difference in initial pH could be from different soil pH in miscanthus plantations. While extruded/retruded miscanthus and miscanthus processed with disc refining had initial pH 6.7–8.0, miscanthus processed with steam explosion had acidic pH of 4.0 (Vandecasteele et al., 2018). Increasing miscanthus portion in substrate mixes raised substrate pH. In substrate mixes of extruded miscanthus, peat and pumice, for fresh substrates (before plant cultivation), when miscanthus proportion increased from 25% to 75%, pH of the mixes increased from 5.0 to 6.0 (Frangi et al., 2012). When the cultivation started, pH of miscanthus substrates increased to neutral–slightly alkaline value of 7.0–7.2 (Frangi et al., 2012). The pH buffering capacity of miscanthus seemed to be low, as amended with a small portion of peat (20%) could reduce substrate pH to normal range of 5.5–6.5 and remained stable (Altland, 2010). Contrarily, the pH in root-zone solution of tomatoes grown in miscanthus growbags maintained around 7.0 when the pH of input fertilization was reduced to 4.5, except the shredded miscanthus decreased gradually its pH 0.5–1.0 unit over the course of cultivation compared to hammermilled substrate (Nguyen et al., 2021).

Initial EC value of fresh miscanthus was in the normal range 0.33–1.4 dS m^{-1} (Nguyen et al., 2021; Vandecasteele, Dias, et al., 2017). Miscanthus might release a significant amount of P and K, as increasing miscanthus proportion in miscanthus-peat-pumice mixes increased the concentration of P and K in extracted substrate solutions (Frangi et al., 2012). Miscanthus field-chips had high organic matter (97%, w/w), high holocellulose: lignin ratio (6.7) with C/N ratio of 178. Water-released nutrients of N, P and K were 29, 12 and 681 mg L substrate^{-1} (Vandecasteele, Dias, et al., 2017). High Si content in miscanthus straw could have potential benefit to tested plants. Compared to standard peat-pine bark-compost mix and other biomass-based substrate mixes, foliar tissue analysis of annual vinca (*Catharanthus roseus*) grown in miscanthus mix showed highest Si content (127.4 mg kg^{-1}) (Altland, 2010).

According to incubation test on fresh substrates, miscanthus straw showed high N immobilization. When incubating miscanthus straw (no processing method mentioned) with N solution (350 mg N L^{-1}) for one week at 37^{o}C, large amount of N applied was immobilized (73–96%). When repeating applying N solution and incubation for six times, amount of N immobilized reduced but still high (52%) (Vandecasteele, Viaene, et al., 2017). The results indicated a strong N immobilization in miscanthus substrate. Vandecasteele et al. (2018) tested the effects of different defibration techniques on N immobilization of miscanthus. Compared to field-chips substrate, processing miscanthus straw with extruder, retruder or disc refining reduced N immobilization (75% vs 33-45%). On the other hand, steam explosion treatment increased N immobilization (55% vs 75%) due to strong increase in water-extractable carbon. Acidification of miscanthus with citric acid or acetic acid reduced N immobilization in incubation trial (from 60% to 20–40%). Substrate pH did not change before and after incubation, so

pH in the range of 2.5 to 4.7 might be effective to reduce N immobilization (Vandecasteele et al., 2018). Whether the pH values would maintain during crop cultivation, thus reduce N immobilization was not investigated in the study. However, adjusting substrate pH below 5 seems quite impractical and could create nutrient imbalance in substrate solution.

3.4.2 Plant growth of nursery shrubs, bedding plants and shrub cuttings in miscanthus

Young plants of shrub species *Ligustrum vulgare* grew better in chopped miscanthus than in extruded miscanthus (Cárthaigh et al., 1997). Regarding fresh plant biomass, substrate mix of peat and miscanthus (60:40, v/v) performed as well as the control substrate (peat: composted bark of 60:40. v/v). Increasing proportion of miscanthus in the substrates reduced plant biomass. Contrarily, young plants of *Hypericum patulum* performed well in both extruded and chopped miscanthus, with a higher proportion of miscanthus in substrate up to 70%. The reasons could be that *Ligustrum* had high demand of nitrogen in which extruded miscanthus might have higher N immobilization than in chopped miscanthus, whereas *Hypercium* preferred more aerated substrate in which extruded miscanthus could advance (Cárthaigh et al., 1997).

The one-year-old plants of cherry laurel (*Prunus laurocerasus* L. 'Rotundifolia') and laurustinus (*Viburnum tinus* L. 'Eve Price') in extruded miscanthus mix (miscanthus: peat: pumice of 25:60:15, v/v) produced much less shoot biomass than the standard peat-pumice substrate (Frangi et al., 2012).

As potting substrate for rooted *Hydrangea arborescens* 'Annabelle', peat: miscanthus (70:30, v/v) with soluble fertilizers and no hydrogel amendment produced shoot weight just after the 100% peat (Pancerz & Bąbelewski, 2019). Increasing the proportion of miscanthus in the mix reduced shoot dry biomass. To root growth, increasing proportion of miscanthus from 0% to 50% increased root biomass, then from 70% to 100% the root biomass reduced.

Miscanthus (hammermilled with screen size of 0.95 cm) can be used as substrate for nursery vinca (miscanthus: peat moss: waste compost = 70:10:20, v/v). Although shoot dry weight and nutrient content in foliar of vinca in miscanthus mix was lower than that on peat-pine bark-compost mix, all plants were marketable after seven-week cultivation (Altland, 2010).

In the eight-week cultivation of *Hibiscus moscheutos*, miscanthus (hammermilled with screen size of 0.48 cm) can be used in the mixes containing 20–60% of miscanthus with at least 20% of pine bark (Altland & Locke, 2011). These ratios showed minimal difference in shoot dry biomass among mix treatments. Foliar SPAD chlorophyll readings were similar among treatments, whereas foliar nutrient analysis showed difference. N content was similar across treatments. The authors concluded that except N content, the other nutrients responded linearly or quadratically to pine bark: miscanthus ratio, but absolute differences in treatments were relatively minor and caused no observable symptoms of nutrient deficiency or toxicity. The authors implied that nutrient uptake of the plants was largely unaffected by substrate types used in the study. Particularly that N immobilization in grass straw-based substrate seemed not to occur. The reason could be that the control released fertilizer was dibbled beneath the

hibiscus liners prior to transplanting. This could place N source near to the reach of plant roots which help them in competing N with microbes.

As rooting substrate, miscanthus: sand (95:5) mix produced highest shoot biomass with average root biomass for the cuttings of semi-hardwood *Taxus* and *Euonymus*, while for the cuttings of softwood *Spiraea* and semi-hardwood *Cotoneaster* a mixture miscanthus with peat (70% miscanthus:25% peat:5% sand) would produce better result (Bąbelewski & Pancerz, 2018).

3.4.3 Strawberry growth in miscanthus

Under the pot cultivation using slow-released N organic fertilizer for three months, strawberries grown in 80% commercial strawberry peat-based substrate mixed with 20% field-chips miscanthus produced lower plant biomass than those grown in 100% commercial substrate (Vandecasteele, Viaene, et al., 2017). Total fruit number of the treatments were comparable, but no data reported on fruit yield. An additional organic fertilizer rate of 2 kg fertilizer m^{-3} miscanthus substrate did not increase plant biomass.

Under the fertigation using ebb and flow system, strawberry plants grown in 80% peat mixed with 20% miscanthus showed lower plant biomass and fruit yield (about 30% less) than those grown in 100% peat in one out of two experiments (Debode et al., 2018). The tested miscanthus substrates were either non-treated (field-chips) or extruded or extruded pre-colonized by *Trichoderma*. Although N immobilization of new field-chips miscanthus (immobilized N of 70%) was significantly higher than extruded miscanthus (immobilized N of 40%) and *Trichoderma* pre-colonized extruded miscanthus (immobilized N of 54%), all three miscanthus substrates performed similarly in the substrates containing 20% miscanthus and 80% peat at cultivation. At the end of cultivation, strawberry roots grown in peat mixed with extruded pre-colonized by *Trichoderma* were consistently fully colonized by *Trichoderma* spp. However, the abundance of *Trichoderma* in the rhizosphere was low in all substrate treatments. Mixing peat with 20% miscanthus substrates did not change bacterial community in the rhizosphere, but change the fungal community structure, with the *Ascomycota* became predominant. In the treatments with miscanthus substrates, the relative abundance of several genera *Cladosporium*, *Pilidium* and *Ilyonectria* containing potentially plant-pathogenic species decreased compared to peat. In post-harvest period, strawberry fruits grown in extruded miscanthus pre-colonized by *Trichoderma* were less susceptible to gray mold (*Botrytis cinerea*) compared to other treatments.

Under pot cultivation using fertigation via drippers, strawberry plants in stand-alone miscanthus field-chips produced comparable marketable fruit yield to those in coir (Kraska & Pude, 2019). Storing miscanthus outdoor (aging) could improve substrate performance as expressed by the highest strawberry yield. Mixing miscanthus with composted spent mushroom substrate at 25-50% or with 50% green compost reduced fruit yield.

3.4.4 Plant growth of tomatoes and cucumbers in miscanthus growbags

In growbags (slabs), substrates are filled in plastic bags (100 x 20 cm length x width, two plants per growbag). Cherry tomatoes grown in growbags containing 80% field-chips miscanthus and 20% wood fibers produced similar yield to those in coir and recycled polyurethane foam (Guo-jing et al., 2002). In drained nutrient solution, pH in miscanthus substrates was slightly alkaline starting from 8 then reducing to 6.5 over the course of cultivation. Nitrate and phosphate concentration in drained solution of miscanthus was lower than in polyurethane foam, but the tomato yield was similar among miscanthus, polyurethane foam and coir. No vegetative growth of tomato plants was described in the study.

In intermediate tomatoes, plants grown in 100% miscanthus (extruded fibers, hammermilled chips or mechanically frayed shreds) showed similar plant growth and fruit yield compared to those in stone wool (Kraska et al., 2018). Besides the genotype *M.* x *giganteus*, genotypes from the species *M. sinensis* and *M. sacchariflorus* 'Robustus' could be used as substrate for tomatoes producing similar yield to stone wool (Nguyen et al., 2021). The authors tested miscanthus as stand-alone substrate with cascade utilization of the used substrates as direct combustion. Given the heating values of used miscanthus substrates were not affected by tomato cultivation, the authors suggested an on-farm bio economy approach that after the primary use as substrate for tomatoes, miscanthus substrate could be subsequently used as solid fuel.

4. Estimation of potential supply capacity of miscanthus as primary feedstock for soilless substrates

As a primary renewable resource, the potential supply capacity of miscanthus depends on land availability and potential yield of miscanthus cultivated on-site. This review estimates the potential supply capacity of miscanthus *M.* x *giganteus* cultivated in Europe harvested in winter-early spring (January-April) for dry matter. To convert mass into volume, the bulk density of miscanthus feedstock is estimated as 0.1 g cm^{-3}.

Based on the dataset on miscanthus yield reported by Li et al., 2018, 156 data inputs for *M.* x *giganteus* harvested in the time frame winter-early spring in Europe were extracted. The mean yield of dry matter was calculated as 15 t ha^{-1} $year^{-1}$. This value represents a medium yield for a miscanthus stand over 20 years of cultivation (Winkler et al., 2020). Allen et al., 2014 estimated that there was 1,300,000 ha land available for energy crops in Europe from agricultural land, in which recently abandoned cropland of 200,000 ha, recently abandoned grassland moving out of agricultural use of 600,000 ha, fallow land in agricultural rotation of 200,000 ha and other underutilized land within the current utilized agricultural area of 300,000 ha. Theoretically, if the area for growing miscanthus takes 1/10 of the total area, the potential supply capacity of miscanthus would be 19.5 Mm^3 annually. Moreover, Gerwin et al., 2018 estimated area of marginal land available for bioenergy crops in Europe using a geographical information system based on soil quality rating system and growing conditions for each bioenergy crop. An area of 5.36 Mha was estimated to be suitable for growing *M.* x *giganteus*. In general, regarding the

availability of land suitable to grow miscanthus in Europe, the potential supply capacity of miscanthus is estimated to be large.

However, the real supply capacity of miscanthus feedstock depends on the land availability on-site and the competition from other industries for miscanthus as feedstock. Despite the multiple potential applications of miscanthus, the area of miscanthus cultivation in Europe is still small (Lewandowski, 2016). According to a survey in 2015, there have been approximately 20,000 ha of miscanthus in Europe, in which about 10,000 ha in the UK, 4,000 ha in Germany, 4,000 ha in France and 500 ha each in Switzerland and Poland (Lewandowski, 2016). In Germany, miscanthus is mainly used for combustion, animal bedding materials and biogas production (Lewandowski, 2016; Winkler et al., 2020). Given the still relatively small market for miscanthus in Europe, the competition seems not be intense, but uncertain in the future. Besides the stated above applications, miscanthus has been studied as potential feedstock for new applications such as polymer composites, concrete materials and particle boards (Moll et al., 2020). Increasing interest in miscanthus applications would increase the competition for the feedstock in the future.

5. SWOT analysis of developing miscanthus into commercial substrate

Based on the summary on miscanthus performance as soilless substrate and potential supply capacity of miscanthus feedstock, the SWOT analysis is conducted to assess the feasibility of developing miscanthus into commercial substrate.

Strengths

- As regional material derived from low-input crop with potential high dry biomass yield, the production cost of miscanthus as primary feedstock for soilless substrate is expected to be low. Because no special machinery is required for harvesting the dry matter, miscanthus cultivation for substrate feedstock is similar to the miscanthus cultivation for combustion described by Winkler et al., 2020. This cultivation system is considered economically viable, with the selling price of miscanthus feedstock of 65–95 € t^{-1} with the production cost of 32–47 € t^{-1} annually.
- Studies on miscanthus as soilless substrate showed a wide range of applications, from rooting substrate to potting substrate for bedding plants, soft fruits (strawberries) and fruit vegetables (tomatoes, cucumbers). Particularly, higher proportion of miscanthus (80–100% miscanthus component) can be used to grow tomatoes and cucumbers in growbags (Kraska et al., 2018; Nguyen et al., 2021). In strawberries, 20% miscanthus mixed with peat provides good carrier for biocontrol agent *Trichoderma* (Debode et al., 2018). The production sectors of soft fruits and fruit vegetables account for high use of soilless substrates.
- As primary material harvested in dry state, it is easier to control the quantity and quality of the feedstock. Due to the high efficient relocation nutrients into the rhizome after senescence, it is expected that the feedstock quality is highly consistent.

- Miscanthus has additional benefits which could potentially enhance substrate performance. High Si content could be released from miscanthus substrate and be absorbed by the crops (Altland, 2010), which could enhance plant resistance to unfavorable abiotic condition. Miscanthus can carry biocontrol agent *Trichoderma* spp., which could enhance suppressive capacity against soil-borne pathogen (Debode et al., 2018).

Weaknesses

- The challenges in improving miscanthus substrate are the low water holding capacity, slightly high pH (7.0–7.5) and high risk of N immobilization. These challenges limit the proportion of miscanthus use in the substrates. Also, substrate stability (i.e., substrate shrinkage) is an issue for crops with long cultivation period. Further modifications (substrate engineering, substrate amendment etc.) must be investigated to improve substrate performance. Due to the additional cost, the tested modifications should be feasible and cost-effective.
- Due to its dryness, miscanthus substrate is dusty and prone to blowing when preparing substrate outdoor.

Opportunities

- Miscanthus cultivation has great socio-economic potential due to its low input demands, high yield and ecosystem services (10–25 t dry matter ha^{-1} annually, carbon sequestration, biodiversity enhancement). Cultivation miscanthus receives support from the policies and community. For example, miscanthus has been accepted to grow in ecological focus area according to the EU Common Agriculture Policies (Emmerling & Pude, 2017; European Commission, 2013).
- The studies on miscanthus agronomy and breeding have been investigating. These studies provide options to improve miscanthus production and feedstock quality, from which soilless substrate sector can benefit.
- Due to the multiple applications of miscanthus such as combustion, animal bedding material, building material, chemical refinery etc., miscanthus after being used as growing substrate is potentially further used in a cascading manner. For example, Kraska et al., 2018 showed that the used miscanthus substrates for tomatoes and cucumbers had similar calorific values to the new miscanthus substrates. The authors suggested that after being used as soilless substrate, miscanthus could be used as direct combustion. Under the economic circular, this approach increases economic value of miscanthus substrate.

Threats

- The multiple applications of miscanthus could add value to the used miscanthus substrates; on the other hand, it also creates competition for feedstock among growing media industry and other industries. Given the still relatively small market for miscanthus in Europe, the competition seems not be intense, but uncertain in the future.

6. Moving forward to increase the use of miscanthus as soilless substrate

Regarding substrate performance, the main issues limiting the use of miscanthus are low water holding capacity, slightly alkaline pH and high risk of N immobilization (i.e. low nitrogen availability for plant roots and also biological instability for long-term cultivation). Substrate properties of miscanthus and peat represent the opposite ends of the spectrum. On the one hand these differences promote mixing miscanthus with peat to enhance aeration and raise pH of peat, but on the other hand they limit the proportion of miscanthus in the substrates, particularly under growing conditions which have been well established for crop cultivation using peat. The published studies mainly investigated the proper ratios of miscanthus in the mixture with peat or other key substrate components. The proportion of miscanthus depends on the crop types (crop demand for nitrogen, aeration and pH) and cultivation techniques (in pots or in growbags, fertilization). Obviously, higher proportion of miscanthus can be used for crops which have moderate demand for nitrogen, prefer aeration and stand wide range of pH.

To reduce N immobilization in miscanthus substrate, the strategies can be categorized into two groups including (1) substrate modifications to create less favor conditions for microorganism growth (e.g., composting, substrate engineering, etc.) and (2) crop management modifications to enhance the access of plant roots to nitrogen (e.g., additional N application, placing N fertilizer near to plant roots, etc.).

Regarding substrate modifications, composting is not an efficient way to treat miscanthus as primary material. Although the miscanthus compost as end product showed potential in growing nursery shrubs (Jensen et al., 2002), the composting process creates barriers to market entry such as sufficient N source to decompose materials efficiently (cost and environmental issues) and high risk of contamination during composting (difficulty in quality control).

Substrate engineering is a potential modification. Proper defibration techniques reduced significantly N immobilization in the incubation test (Vandecasteele et al., 2018), but not in plant test (Debode et al., 2018). To define proper processing methods, further investigation should focus on feasible techniques to engineer miscanthus substrates for better substrate performance. Further investigations should also focus on feasible changes in crop management to enhance nutrient availability to plant roots. For example, minimal additional N should be applied to compensate for N used by the microbes, particularly in the advance of close soilless culture loops which allowing nutrient recycle.

To secure supply capacity of the feedstock, future studies should also explore possible cascade utilization of used miscanthus substrates. The findings would add market values to the used miscanthus substrates and reduce possible competition for the feedstock from other industrial applications. For example, Kraska et al., 2018 first showed that miscanthus could have primary utilization as soilless substrate for tomatoes and cucumbers. As the heating values of used miscanthus were comparable to the new feedstock, used miscanthus substrates could be served as feedstock for direct combustion and the resulting ash for soil amendment.

7. Conclusions

This review evaluates the feasibility of developing miscanthus into commercial soilless substrate. Although showing potential as soilless substrate for a wide range of applications, the adoption of miscanthus as growing substrate is still at research level. Two main impediments are (i) the challenges in substrate performance as low water holding capacity, slightly high pH and high risk of N immobilization which limit the proportion of miscanthus in the substrates; (ii) the limited availability of miscanthus materials on-site due to the still small market for miscanthus in Europe.

To develop miscanthus into commercial soilless substrate, further studies should focus on substrate modifications and management modifications to enhance nutrient availability for plant roots. The promising research findings should be then scaled up into commercial conditions. Once the reliable and consistent performance of miscanthus is proved under commercial growing conditions, it will create new market for miscanthus as feedstock for soilless substrate. Given potential large land availability for growing miscanthus and relatively small market for miscanthus in Europe, the competition for feedstock seems not be intense at present, but uncertain in the future. Cascade utilization of used miscanthus substrate should be investigated, and thus both increase resource efficiency and reduce feedstock competition.

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Chapter 3

Miscanthus as stand-alone substrate in growbags for tomatoes with cascade utilization as direct combustion

Highlights

- pH, electrical conductivity and nitrate concentration in root-zone solution were different among miscanthus substrates, stone wool and coir.
- Plants in miscanthus substrates produced comparable yield to those in stone wool, but lower than those in coir.
- Miscanthus genotypes, substrate amendment with elemental sulfur or $Ca(NO_3)_2$ showed no effect on plant yield.
- In soilless tomato culture, miscanthus performed consistently with the benefit of subsequent utilization as solid fuel.

Abstract

Dry biomass of *Miscanthus* spp. is a promising renewable feedstock for soilless substrate. This study aims to test the feasibility of using miscanthus as stand-alone substrate to grow tomatoes in growbags with a cascade utilization as direct combustion. In the experiment in 2018, the effects of different miscanthus genotypes (*M.* x *giganteus*, *M.* '*Robustus'* 24, *M. sinensis* 21) under two nutrient regimes (reference vs modified pH and nutrient concentration of the input) on root-zone pH, electrical conductivity (EC), plant performance and combustion quality of the used substrates were tested. In the experiment in 2019, the effects of substrate processing (hammermilled and shredded of *M.* x *giganteus*) and substrate amendments with elemental sulfur or $Ca(NO_3)_2$ on root-zone pH, EC, nitrate concentration and plant performance were investigated. Commercial growbag substrates as stone wool (Grodan) and coir (Jiffy) were included. The results showed that tomatoes in miscanthus substrates produced consistent fruit yield over two experiments. Miscanthus substrates showed higher pH and lower nitrate concentration than stone wool and coir. Fruit yield of tomatoes in miscanthus were similar to that in stone wool, but lower than in coir. Fruit yield was mainly related to fruit loss by blossom-end rot. Treatments did not show effect on pH and EC in root-zone solution, except shredded miscanthus had lower pH only in the experiment 2018. Miscanthus genotypes showed effects on fruit quality of tomatoes and combustion quality of the used substrates. Calorific values of used miscanthus substrates were comparable to the new ones. In conclusion, in soilless tomato cultivation, miscanthus can replace stone wool with a benefit of subsequent utilization as solid fuel.

Key words: growing media, miscanthus genotypes, blossom-end rot, rockwool, coir

Abbreviations: BER (blossom-end rot), EC (electrical conductivity), DAT (days after transplanting)

(in preparation as a manuscript to be submitted to journal Scientia Horticulturae)

1. Introduction

Tomato is one of the most important fruit vegetables produced globally (Shahbandeh, 2021). Large production of tomatoes in Europe is cultivated in soilless culture using pre-shaped growbags filled with inert mineral material named stone wool (Kubota et al., 2018). The production process of new stone wool and disposal of used substrates have negative impacts on environment (Gruda, 2019), which has led to demands for sustainable alternative materials. Several materials have been tested to grow tomatoes in soilless culture such as polyurethane foam (Benoit & Ceustermans, 2004; Guo-jing et al., 2002), wood fibers (Gruda & Schnitzler, 2004), *Sphagnum* biomass (Dannehl et al., 2015), pine bark amended cotton gin compost (Jackson et al., 2019) and coir (Domeño et al., 2009; Xiong et al., 2017). Among them, only coir has been a commercial stand-alone substrate. However, this material may not be the best option for the temperate regions because of its long distance transportation from the tropics (Barrett et al., 2016). The challenge for European countries is to search for regional renewable resources from which substrates can be produced in sufficient quantity with economic cost and minimal environmental impacts as well as substrate performance must be reliable and consistent.

Miscanthus (*Miscanthus* spp.), a perennial rhizomatous C4 crop, has been cultivated widely in Europe and North America mainly for solid fuels. Miscanthus is one of the most productive land plants in temperate climates. Yields can reach up to 55 t dry mater ha^{-1} and in many countries yields above 20 t dry matter ha^{-1} are reported (Heaton et al., 2010). Plant senescence occurs in winter time resulting in dry plant biomass in spring time. Without additional drying, miscanthus can be chopped or shredded into small particles for use as soilless substrate.

Miscanthus was studied as growbag substrate for tomatoes showing comparable yield to polyurethane foam (Guo-jing et al., 2002) and stone wool (Kraska et al., 2018). Guo-jing et al., 2002 tested miscanthus substrate comprising of 80% miscanthus and 20% wood fiber. In drained nutrient solution of miscanthus substrate, pH was slightly alkaline starting from 8 then reducing to 7 over the course of cultivation. Nitrate and phosphate concentration in miscanthus were lower than in polyurethane foam, but the tomato yield was similar among miscanthus, polyurethane foam and coir. No vegetative growth of tomato plants was described in the study. In the study from Kraska et al., 2018, miscanthus was first studied as stand-alone substrate, i.e. miscanthus as sole component in growbags, with additional benefit as direct combustion of the used substrates. Shredded, hammermilled and extruded miscanthus were tested. Plant growth expressed as stem length and fruit yield were comparable among all substrates.

Besides the two studies on miscanthus as growbag substrate, miscanthus has been studied as container substrate for nursery shrubs (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012), wood cuttings (Bąbelewski & Pancerz, 2018), strawberries (Debode et al., 2018; Kraska & Pude, 2019; Vandecasteele et al., 2017). While miscanthus could be used up to 80-100% in growbags showing no yield reduction, increasing proportion of miscanthus in container substrates lead to plant growth reduction. Reported challenges of miscanthus substrate include low water holding capacity (Altland &

Locke, 2011; Cárthaigh et al., 1997), slightly high pH than the recommended range for greenhouse crops (Altland & Locke, 2011; Frangi et al., 2012) and risk of nitrogen (N) immobilization thus substrate shrinkage (Cárthaigh et al., 1997; Debode et al., 2018; Frangi et al., 2012; Vandecasteele et al., 2017; Vandecasteele et al., 2018). To develop miscanthus into growbag substrate for tomatoes, substrate performance should be further investigated focusing on the reliable and consistent performance.

All studies on miscanthus as soilless substrates used *M.* x *giganteus*, the most commonly used genotype in the temperate regions, not taking into account that other miscanthus species like *M. sinensis* or *M.* '*Robustus'* might be advantageous. Miscanthus comprises more than 16 species which have different biomass partitioning to leaf and stem, flowering, senescence and different chemical compositions (Brosse et al., 2012; E. Jensen et al., 2017; Monti et al., 2008). These differences in feedstock could have an effect on substrate performance. Moreover, the feasibility of using other species as *M. sinensis* or *M.* '*Robustus'* as soilless substrates would also diversify miscanthus feedstock, which has been solely investigated for *M.* x *giganteus*.

Elemental sulfur has been used to lower pH of compost and biomass substrates (Altland & Krause, 2010; Costello & Sullivan, 2014). Elemental sulfur reduced substrate pH in switchgrass, but the high application rate of 1 lb $yard^{-3}$ (equivalent to 0.6 kg m^{-3}) or greater reduced pH to values lower than 5.5 (Altland & Krause, 2010). A practical approach to reduce N immobilization in substrates is to apply additional N amount to compensate for N taken up by microorganism (Handreck, 1993; Jackson et al., 2009). Soilless cultivation of tomatoes using fertigation allows to acidify substrates and apply additional N via modifying pH and nutrient concentrations in the nutrient input. Prior to transplanting, substrates in growbags must be fully wetted. Miscanthus particles can absorb water and expand; therefore, instead of using water, wetting miscanthus substrates with N solution might be a practical way to amend miscanthus substrates with additional N.

In the context of circularity, possible ways of further use of substrate should be considered. As a feedstock for solid fuel, miscanthus substrate could be still a promising feedstock for combustion after being used as soilless substrate. Given the heating value of used miscanthus substrates were not affected by tomato cultivation, Kraska et al., 2018 suggested an on-farm bio-economy approach that after the primary use as substrate for tomatoes, miscanthus substrates could be subsequently used as solid fuel. One limitation in using used miscanthus substrates for direct combustion is the high ash content.

The aim of this work is to introduce miscanthus substrate as stand-alone growbag substrate for tomatoes with an additional benefit as direct combustion of the used substrates. Tomato growth were investigated in two experiments in 2018 and 2019. The experiment in 2018 tested the effects of miscanthus genotypes as substrates under specific pH and nutrient strength of input solution on (i) pH and electrical conductivity (EC) in root-zone solution, (ii) on plant performance and (iii) on combustion quality of used substrates. The experiment in 2019 tested the effects of substrate processing (hammermilled and shredded) and substrate amendments with elemental sulfur or $Ca(NO_3)_2$ on (i) pH and EC in root-zone solution and (ii) on plant performance. The tomato growth in 2018 and 2019 was

used to evaluate the consistent performance of miscanthus substrates. A part of the results of the experiment 2018 was reported in the journal *Acta Horticulturae*, a proceeding of the International Society of Horticultural Science III International Symposium on Growing media, Composting and Substrate analysis in Milan, 2019 (Nguyen et al., 2021).

2. Materials and methods

Experimental setup

Both experiments were conducted in the same compartment of a multi-span Venlo greenhouse at Campus Klein-Altendorf, University of Bonn (Rheinbach, Germany) in 2018 and 2019. The compartment has an area of 224 m^2 with six hanging gutters, 40 growbags per gutter. Growing practices and methodology were the same for both experiments, except where noted.

Tomato seeds (intermediate cultivar 'Lyterno RZ F1', Rijk Zwaan) were sown into stone wool plugs, then cultivated in stone wool cubes for 30 days. The cubes were then transplanted onto growbags (100 x 20 cm length x width, two plants per growbag) containing stone wool (Rockwool, Grodan Grotop master), coconut coir (Jiffy High yield Growbag) or different miscanthus substrates (2 kg of miscanthus substrate per bag).

Miscanthus stands cultivated at Campus Klein-Altendorf were harvested at ground level using a forage harvester (Champion C1200, Kemper, Germany) in April 2017. The harvested materials were stored in a protected barn. In January 2018 and 2019, one month before the transplanting, harvested miscanthus materials were processed further using either a hammermill (Type BHS100, Buschhoff, Germany) with screen size of 15 mm resulted in miscanthus chips (C15) or a mechanical fraying facility (Type ZF 140/B4, Eirich, Germany) with screen size of 5 mm resulted in miscanthus shreds (S5).

Before the transplanting, all growbags were placed on the four inner gutters in a randomized block design. Growbags were watered slowly from drippers (100 ml per hour, about 4 to 5 L water per bag) to saturate substrates. After that, a drainage cut (about 7 cm) was made in the middle at one side of growbags (about 4 cm above the bottom). The nutrient solution was applied via drippers placed near the plant stems (one dripper per plant). Composition of nutrient solution was listed in the Supplementary (Table S3.1). Tomato plants were cultivated in 'leaning and lowering' system for commercial greenhouse tomatoes. Suckers, i.e. side shoots, and lower leaves were pruned occasionally to balance vegetative and generative growth. Pollination was done by bumblebees. Each fruit truss was pruned to six fruits to allow homogenous fruit development. Blossom-end rot fruits (BER) were monitored weekly and pruned as soon as detected. Tomatoes were harvested as a whole truss once per week from May to September. Detailed practices in each experiment were listed in the Supplementary (Table S3.2, S3.3).

Experiment 2018: Effects of miscanthus genotypes as growbag substrates on tomato growth

Miscanthus substrates derived from three miscanthus genotypes including *M.* x *giganteus*, *M. sinensis* 21 and *M.* '*Robustus'* 24 (Table 3.1). Two nutrient regimes were applied: regime 1 as

"reference" nutrient solution had pH 5.5 and EC 2.6 dS m^{-1} (Table S3.1), regime 2 as "modified" nutrient solution had pH 4.5 and EC 3.0 dS m^{-1}, with increased concentration of all nutrients. Due to the system management applied for all substrates, in regime 2, pH 4.5 was applied from day 15 to day 72 after transplanting and then adjusted back to 5.5 as pH in stone wool kept reducing. EC 3.0 dS m^{-1} was applied from day 36 to day 219 (two weeks before the end of cultivation). In the last two weeks before the cultivation end, only water was applied in the regime 2 to reduce ash content in the substrates.

Table 3.1 List of substrates used in the experiment in 2018

Substrate	Source
Stone wool	Rockwool, Grodan Grotop master
Coir	Coconut coir, Jiffy High yield Growbag, double-layered
GigS5	*Miscanthus* x *giganteus*, shreds, mechanical fraying facility screen size of 5 mm
GigC15	*M.* x *giganteus*, chips, hammermill screen size of 15 mm
RobC15	*M.* '*Robustus'* 24, chips, hammermill screen size of 15 mm
SinC15	*M. sinensis* 21, chips, hammermill screen size of 15 mm

Substrate parameters

The intact culms from three miscanthus genotypes were cut in April, then separated remaining leaves from the stem for biomass (4 replicates, each replicate consists of 5 culms).

For processed miscanthus substrates, particle size distribution, bulk density, pH, EC and pH buffering capacity were tested. Particle size of substrates was investigated with dynamic image analysis using Particle Analyzer CamSizer P4 (Microtrac Retsch GmbH, Germany). Substrates were oven-dried at 105°C for 2 days, then divided into subsamples (4 replicates, about 25 g each) with the Sample Divider (Microtrac Retsch GmbH, Germany). Substrates were transported along a vibrating feeder until they fell freely into a measurement shaft where two high-speed cameras capturing their images. The images were analyzed with CamSizer software to characterize size of particle projection based on projected volume. Bulk density expressed as fresh weight per volume was measured by filling fresh substrates (moisture content about 10%) into a one-liter cylinder, tapping on a working bench for six times, then levelling up the surface and measuring fresh weight of substrate. pH and EC were measured using saturation method, by weighing 10 g of fresh substrates into a vial, then slowly adding demineralized water until saturation, setting aside for one hour then measuring pH and EC in the suspension using a pH meter (pH 3000, STEP Systems GmbH, Germany) and an EC meter (FSEC20, MMM Tech Support GmbH & Co. KG, Germany). pH buffering capacity of miscanthus substrates was assessed by adding nutrient solution at pH values of 2, 5 and 9 to fresh substrates. Two g of miscanthus substrates were added with 20 ml of the same nutrient solution used for tomatoes with pH adjusted to pH 2, 5 and 9 using HNO_3 38% and NaOH 10%. pH values were measured directly in substrate solution at 0, 1, 2.5, 4, 6, 22.2, 24 and 30.8 hours after nutrient addition (4 replicates).

During the tomato cultivation, pH and EC values in substrate solution were monitored regularly (4 replicates).

Vegetative growth

Vegetative growth of tomato plants was monitored at three development stages including early stage (from transplanting to 60 days after transplanting (DAT), early stage of harvest period (around 100 DAT) and middle of harvest period (around 130 DAT). Without destructive sampling, vegetative parameters included stem length and stem circumference (measured below the first fruit truss, i.e. mostly between the node 8 and 9), removal sucker biomass from the first pruning and biomass of pruned leaves. Leaf biomass and leaf area of single leaf were calculated from total biomass and area of pruned leaves and number of pruned leaves at each sampling date. Leaf area was measured with an area meter (LI-3100C Area Meter, LI-COR). Leaf chlorophyll was measured with the SPAD 502 plus Chlorophyll meter (Minolta Camera Co. Ltd., Japan). SPAD value of a plant was calculated as the average of values measured at the lower leaf and the top leaf under the highest truss. The measurement dates of each vegetative parameter were listed in the table S3.2.

Fruit yield and quality

Tomatoes were harvested once per week followed by their truss order until the plants reached the truss 20 and cumulative fresh yield was calculated (12 replicates, each replicate consists of one plant). Number of BER fruits was recorded by truss order and cumulatively calculated. For fruit quality, the first fruits from truss one and truss seven were collected to measure fruit biomass and total soluble solids (TSS, ^{o}Brix) using the digital refractometer DBR45.

Combustion quality of substrates

At the end of cultivation, substrates from each growbag were collected separately and dried in oven at 105^{o}C for two days. Sub-samples of the used substrates were collected from each growbag (four replicates) for combustion quality. New and used miscanthus substrates were analyzed for calorific value and ash content. Gross calorific values were analyzed with the C 200 calorimeter (IKA-Were GmbH & CO. KG, Germany) using the isoperibolic measurement method. Ash content was measured by weight difference after heating substrate samples in a muffle furnace (L 9/11/B180, Nabertherm GmbH, Germany) at temperature of 550^{o}C (± 10^{o}C) for 4 h.

Experiment 2019: Effects of substrate amendment with $Ca(NO_3)_2$ and elemental sulfur on tomato growth

Miscanthus substrates GigC15 and GigS5 were amended with $Ca(NO_3)_2$ or elemental sulfur. At filling substrate into growbags, substrates were mixed with elemental sulfur powder (Netzschwefel Stulln, 80%) at the rate 0.3 kg elemental S per m^3 of substrate. For the treatments with $Ca(NO_3)_2$, five days before transplanting, miscanthus growbags were pre-wetted with $Ca(NO_3)_2$ solution at the rate of 5 kg $Ca(NO_3)_2$ per m^3 of substrate. Other growbags were pre-wetted with water as usual.

Table 3.2 List of substrates used in the experiment in 2019

Substrate	Source
Stone wool	Rockwool, Grodan Grotop Master
Coir	Coconut coir, Jiffy High yield Growbag double-layered
GigC15	*Miscanthus* x *giganteus*, chips 15 mm
GigC15b	*M.* x *giganteus*, chips 15 mm, buffered with $Ca(NO_3)_2$ (5 kg $Ca(NO_3)_2$ per m^3 substrate)
GigC15s	*M.* x *giganteus*, chips 15 mm, amended with elemental sulfur (0.3 kg S^0 per m^3 substrate)
GigS5	*M.* x *giganteus*, shreds 5 mm
GigS5b	*M.* x *giganteus*, shreds 5 mm, buffered with $Ca(NO_3)_2$ (5 kg $Ca(NO_3)_2$ per m^3 substrate)
GigS5s	*M.* x *giganteus*, shreds 5 mm, amended with elemental sulfur (0.3 kg S^0 per m^3 substrate)

Chips 15 mm, shreds 5 mm: substrate processed with a hammermill through screen size of 15 mm, or with a mechanical fraying facility through screen size of 5 mm, respectively.

Substrate parameters

pH, EC and NO_3^- in root-zone solution were monitored over the course of cultivation. NO_3^- concentration was measured with the NO_3^- meter (LAQUAtwin, Horiba Scientific, Japan).

Early vegetative growth, fruit yield and fruit quality

During 60 DAT, early vegetative growth of tomato plants was assessed via sucker biomass, stem length, stem circumference and pruned leaf biomass. First sucker biomass was measured at 15 DAT. Stem length and stem circumference were measured at 31 DAT. Lower leaves were pruned at 41, 51 and 58 DAT. Fruit yield, fruit loss by BER and fruit quality were measured as described for the experiment in 2018, except the fruit quality was measured in truss one and truss five. Sampling schedules were in the Supplementary (Table S3.3).

Statistical analysis

Differences in mean among treatments were analyzed with one-way ANOVA test ($p \leq 0.05$) and followed by Tukey's HSD post-hoc test ($p \leq 0.05$). The statistical analysis was carried out using the R software version 4.0.2. The graphs were prepared using the package ggplot2 in R.

3. Results

Experiment 2018: Effects of miscanthus genotypes as growbag substrates on tomato growth

Properties of miscanthus feedstock and processed miscanthus substrates

Three miscanthus genotypes showed different culm morphology at harvest (Table 3.3). *M.* x *giganteus* had the highest stem biomass and higher stem proportion in the harvested culms. Stem biomass of *M.* '*Robustus'* was lower than that of *M.* x *giganteus* and *M. sinensis* with a factor of five and three times, respectively. Both *M. sinensis* and *M.* '*Robustus'* had lower stem: leaf ratio than that in *M.* x *giganteus*.

Table 3.3 Culm morphology of three miscanthus genotypes at spring harvest

Miscanthus genotype	Stem biomass (g)	Leaf biomass (g)	Stem:leaf ratio
M. x *giganteus*	175.1 ± 30.7 a	36.0 ± 4.4 a	4.8 ± 0.4 a
M. 'Robustus' 24	34.2 ± 3.1 c	10.8 ± 1.6 b	3.2 ± 0.3 b
M. sinensis 21	109.0 ± 12.9 b	35.3 ± 4.5 a	3.1 ± 0.2 b

Different lower case letters indicate statistically significant differences in means among three miscanthus genotypes at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 4, each replicate consists of 5 culms)

In both particle dimensions, the hammermilled substrates of *M.* x *giganteus* (GigC15) and *M. sinensis* (SinC15) had similar proportion of fine particles (< 0.5 mm), whereas *M.* '*Robustus'* (RobC15) had the highest fine particle proportion (Table 3.4). Their fractions of medium and coarse particles were different, particularly in particle width. The fractions of medium particles (0.5–2.0 mm) of the genotypes were in inverse relation to their stem biomass, where the fractions of coarse particles (> 2 mm) showed positive relation (Table 3.3, 3.4). Having the smallest stem biomass, RobC15 substrate had the highest fraction of fine and medium particles and the lowest fraction of coarse particles. In particle width, shredded *M.* x *giganteus* (GigS5) had similar fraction of fine particles, higher fraction of medium particles and lower fraction of coarse particles compared to hammermilled *M.* x *giganteus* (GigC15) (Table 3.4).

Table 3.4 Particle size distribution of miscanthus substrates

Substrate	Fraction of particle width (%)			Fraction of particle length (%)		
	Fine	Medium	Coarse	Fine	Medium	Coarse
GigS5	16.5 ± 1.6 ab	65.0 ± 0.9 a	18.5 ± 1.9 c	6.7 ± 1.2 b	16.7 ± 0.4 a	76.6 ± 1.0 a
GigC15	15.8 ± 1.2 bc	49.5 ± 1.4 c	34.7 ± 0.7 a	8.1 ± 0.7 b	14.1 ± 0.4 b	77.8 ± 1.1 a
RobC15	19.9 ± 2.5 a	64.3 ± 1.2 a	15.8 ± 1.2 d	13.1 ± 3.3 a	16.3 ± 0.8 a	70.6 ± 2.7 b
SinC15	13.0 ± 0.9 c	60.4 ± 0.4 b	26.7 ± 0.6 b	7.1 ± 0.7 b	15.7 ± 0.3 a	77.2 ± 0.8 a

Particle size classes including fine (< 0.5 mm), medium (0.5–2.0 mm) and coarse (> 2.0 mm) (Drzal et al., 1999).
Different lower case letters indicate statistically significant differences in means among four miscanthus substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 4, each replicate consists of 25 g dried substrate).
GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Miscanthus substrates had initial pH in the range 6.0–6.3, except SinC15 with highest pH 6.9 (Table 3.5). All miscanthus substrates increased their pH when adding nutrient solution with pH 2 and 5, and decreased their pH when adding nutrient solution with pH 9 (Figure 3.1). pH increase in SinC15 was higher than that in GigC15, RobC15 and GigS5, that could be of higher initial pH in SinC15.

Table 3.5 Bulk density, pH and electrical conductivity (EC) of substrates

Substrate	Bulk density (kg m^{-3})	pH	EC (dS m^{-1})
Stone wool	n.d.	8.21 ± 0.12 a	0.06 ± 0.02 b
Coir	n.d.	5.23 ± 0.10 e	1.28 ± 0.49 a
GigS5	174.4 ± 6.8 a	6.18 ± 0.02 d	1.41 ± 0.18 a
GigC15	142.8 ± 2.6 b	6.32 ± 0.03 c	1.32 ± 0.11 a
RobC15	144.2 ± 3.8 b	6.06 ± 0.03 d	0.92 ± 0.10 a
SinC15	116.2 ± 2.8 c	6.91 ± 0.04 b	1.01 ± 0.09 a

n.d.: not determined for commercial pre-shaped substrates. Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 4). Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

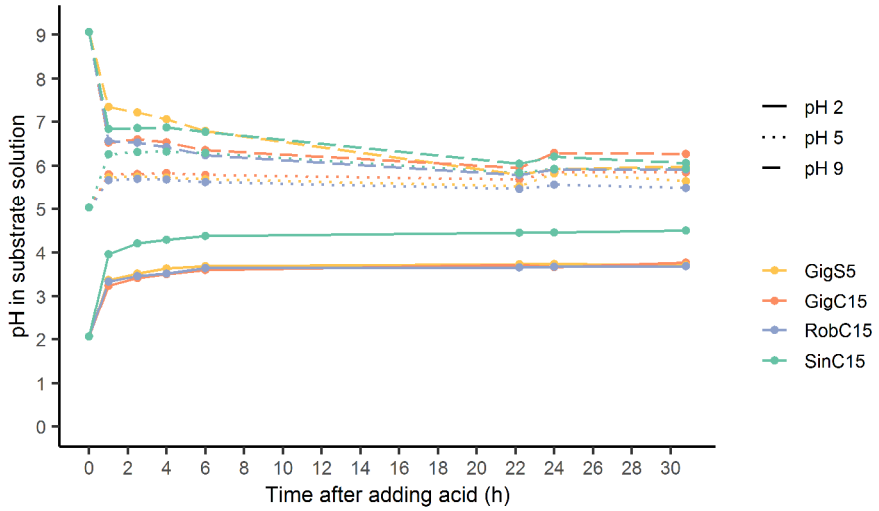

Figure 3.1 pH evolution in substrate solution of miscanthus when adding nutrient solutions with pH 2, 5 and 9. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively, (n = 4).

pH, EC in root-zone solution in tomato cultivation

Tomato plants grown in all substrates shared similar developmental pattern. The first anthesis, i.e. full-blossom of the first flower in the first truss, occurred in the period from 20 to 34 DAT. The harvest of the first truss occurred from 90 to 104 DAT.

Over the course of cultivation, in both regimes, pH evolution of all substrates followed a same pattern: pH values increased during strong vegetative growth and reached the highest values around 35 DAT, then decreased gradually when generative growth started and reached the lowest values during the harvest period of truss 1 to truss 8 (90–145 DAT) (Fig 3.2). The fluctuation ranges of pH values were different among substrates, following this descending order of range as stone wool > coir > miscanthus substrates. Stone wool had the widest pH range (3.8–7.4), pH increased from 5.8 to 7.4 during vegetative growth, then decreased and maintained around pH 3.8 to 5.0. Coir showed narrower fluctuation range (5.0–7.1) with pH maintaining around 5 to 6. Miscanthus substrates showed the narrowest range (6.1–7.8) with pH maintaining around 7. No significant genotype effect on substrate pH was observed. Among miscanthus substrates, pH in GigS5 decreased to lowest values towards the end of cultivation. However, the differences in pH among miscanthus substrates were not significant, except in regime 2 at 46, 96, 103 and 117 DAT, pH in GigS5 was significant lower (0.8–1.0 pH unit) than pH in GigC15.

EC values of all substrates increased gradually and reached the plateau at around 90 to 145 DAT (Figure 3.2). After that, due to the high EC values in stone wool growbags, at 154 DAT water was applied to all growbags for one day to reduce EC values in stone wool growbags. Again, the fluctuation range of EC value was different following the descending order of the range as stone wool > coir > miscanthus substrates. EC values in stone wool growbags increased from 1.6 to 6.5 dS m^{-1}, while EC in coir growbags increased from 2.0 to 4.9 dS m^{-1}. All miscanthus substrates stayed around 2.0 dS m^{-1} with

only small variability. Among miscanthus substrates, EC in GigS5 substrates in regime 2 were slightly higher with values of 2.5 to 3.0 dS m^{-1}, however, the differences were not statistically significant, except in regime 2 at 196 DAT, GigS5 was significantly higher 1.5 unit of EC than other miscanthus substrates.

Because of no replication of input regime, pH and EC evolution of each substrate between two regimes was plotted without statistical test (Figure 3.3). At the early vegetative growth, i.e. before the vigorous development of tomato fruits (around 35 DAT), all substrates showed similar pH, EC values between two regimes. This indicated that at this stage, tomato plants seemed to strongly control root-zone pH and EC. After the phase of vigorous vegetative growth, changes in root-zone pH and EC seemed to be interacted between tomato plants and substrates. Coir and hammermilled miscanthus substrates (GigC15, RobC15 and SinC15) showed similar pH and EC values between two regimes. Stone wool and GigS5 had lower pH and higher EC in regime 2 in generative growth phase.

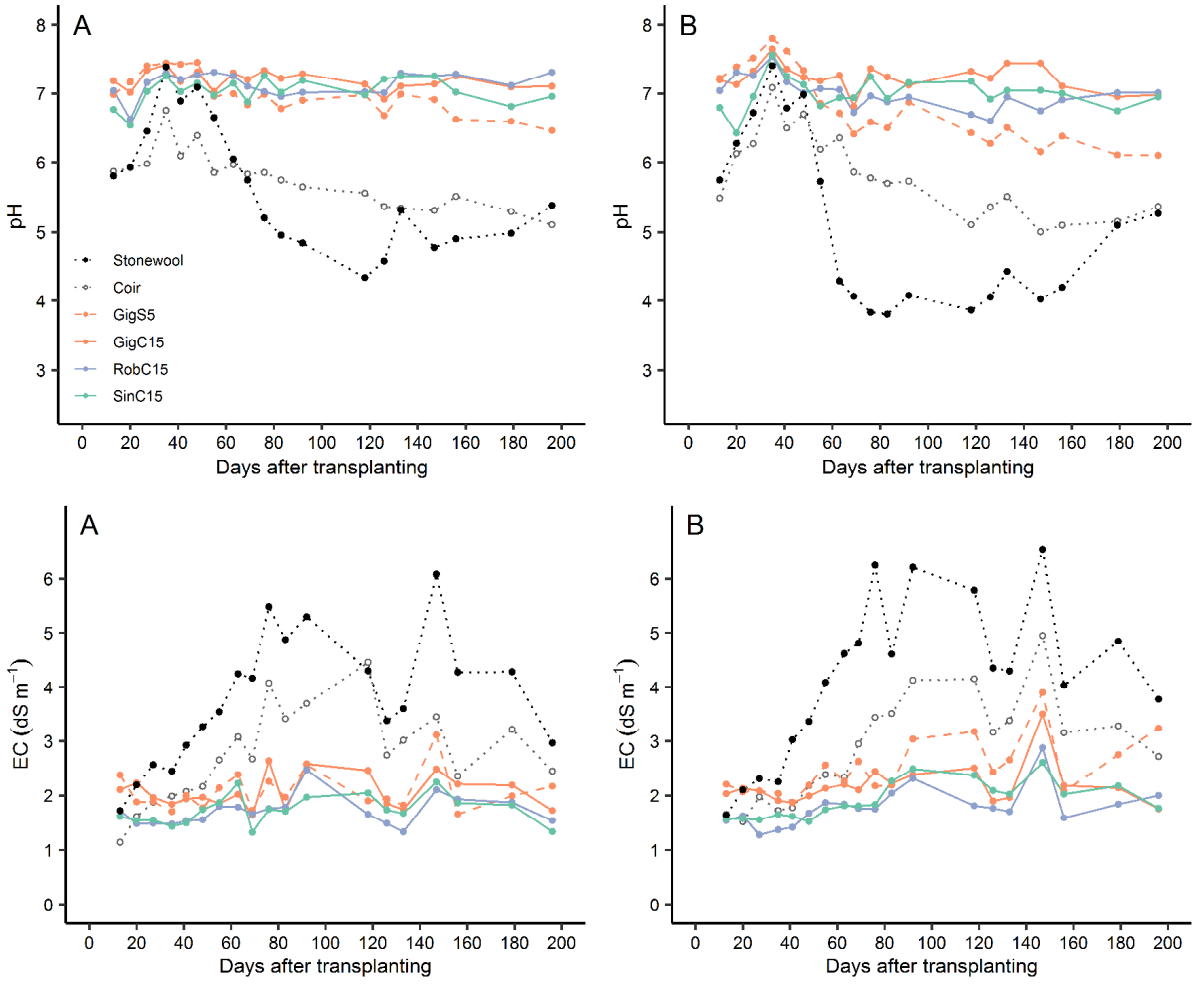

Figure 3.2 pH and EC values in root-zone solution of tomatoes grown in different substrates under (A) input regime 1 (pH 5.5, EC 2.6 dS m^{-1}) and (B) input regime 2 (pH 4.5, EC 3.0 dS m^{-1}) (n = 4).
Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M. 'Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

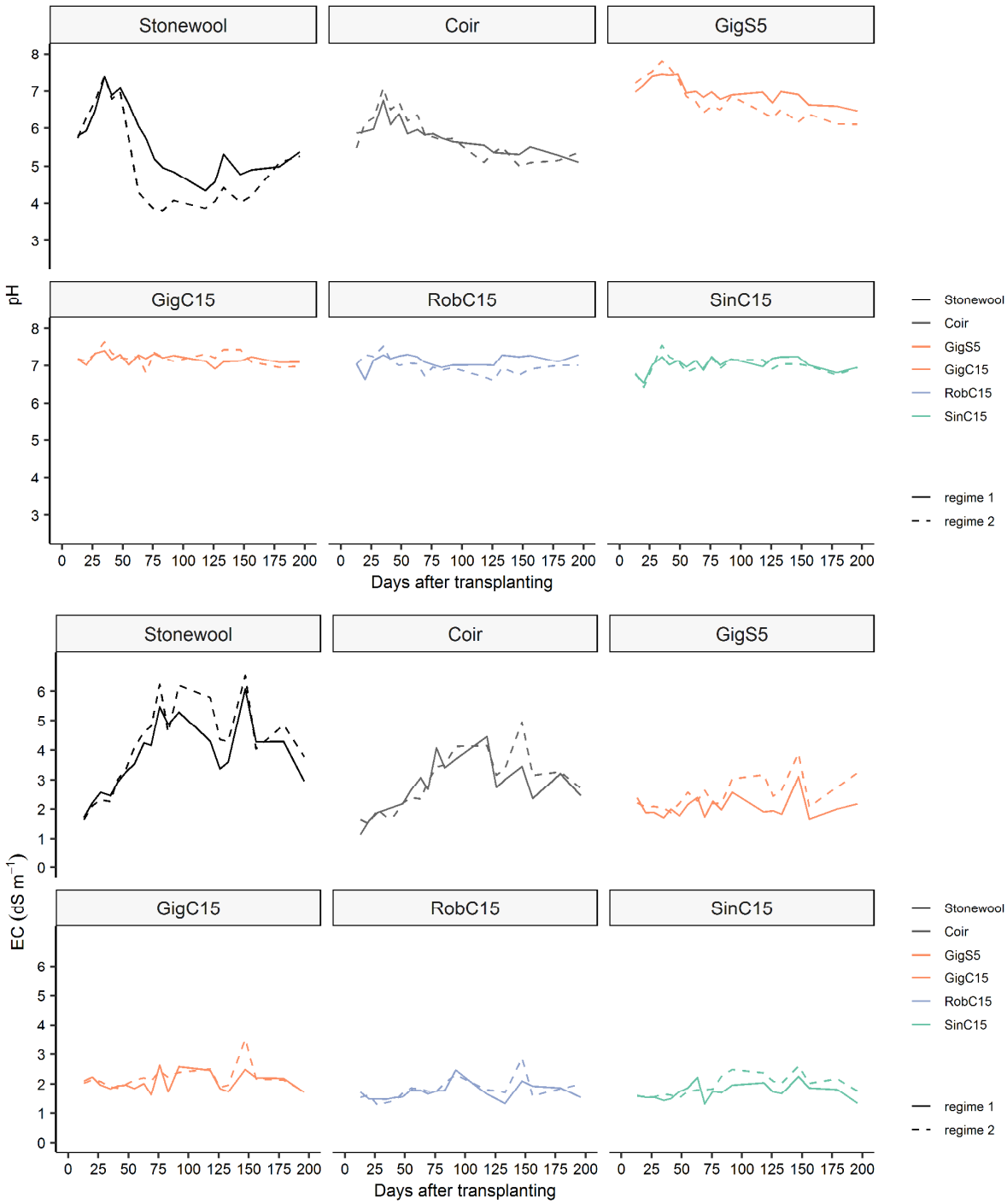

Figure 3.3 pH and EC change in root-zone solution of each substrate between two nutrient regimes: regime 1 (pH 5.5, EC 2.6 dS m^{-1}) and regime 2 (pH 4.5, EC 3.0 dS m^{-1}).
Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus*' 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Vegetative growth

In regime 1, at the early growth stage (around the onset of 1st anthesis, 20 DAT), expressed by stem circumference and fresh biomass of suckers at the first pruning, plants in stone wool and coir showed more vigorous vegetative growth than those in SinC15, but comparable to those in other miscanthus

substrates (Figure 3.4, table 3.6). During the time of vigorous vegetative growth (around 30–50 DAT), expressed by stem circumference, biomass and leaf area of pruned leaves, plants in all miscanthus substrates showed lower vegetative growth than those in stone wool and coir (Table 3.6, 3.7). Among miscanthus substrates, plants in SinC15 produced smallest stem (Table 3.6). After the peak of vegetative growth to the first harvest period (around 100 DAT), vegetative growth of plants in stone wool and coir was still more vigorous than those in miscanthus substrates, but the degree of difference reduced towards the start of fruit harvest (Table 3.6, 3.7). Stem length and SPAD value did not show difference among substrates, except the SPAD value at 36 DAT showing highest SPAD value in SinC15 (Table 3.6, 3.8). In regime 2, vegetative growth of substrates showed similar pattern in regime 1, but lower degree of difference.

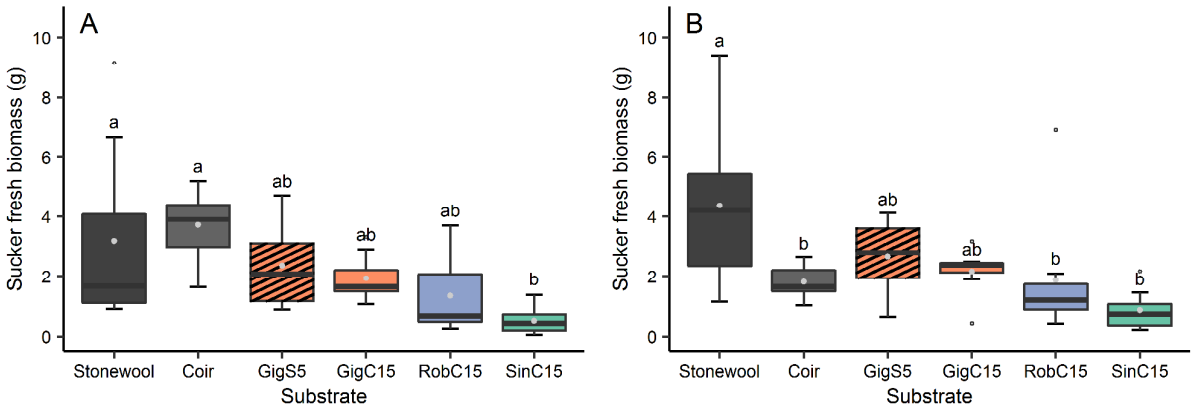

Figure 3.4 Fresh biomass of suckers at the first pruning at 19 days after transplanting in (A) input regime 1 (pH 5.5, EC 2.6 dS m^{-1}) and (B) input regime 2 (pH 4.5, EC 3.0 dS m^{-1}).
The gray dots represent for the means. The dots out of the whisker range are the outliers. Different lower case letters indicate statistically significant differences among substrates (Tukey's HSD, $p \leq 0.05$, $n = 8$). Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Table 3.6 Stem circumference and stem length of tomato plants over the course of cultivation

Substrate	Stem measurement at				
	22 DAT	42 DAT	105 DAT	133 DAT	175 DAT
Stem circumference (cm)					
Regime 1 (pH 5.5, EC 2.6 dS m^{-1})					
Stone wool	3.7 ± 0.6 ab	5.0 ± 0.3 a	6.4 ± 0.4 a	6.7 ± 0.5 a	6.4 ± 0.5 a
Coir	4.0 ± 0.3 a	5.2 ± 0.2 a	6.6 ± 0.4 a	6.3 ± 0.5 ab	6.1 ± 0.4 ab
GigS5	4.0 ± 0.3 a	4.9 ± 0.3 a	6.3 ± 0.3 ab	6.3 ± 0.4 ab	6.0 ± 0.4 ab
GigC15	3.8 ± 0.3 a	4.8 ± 0.1 a	6.1 ± 0.3 ab	6.3 ± 0.4 ab	6.0 ± 0.4 ab
RobC15	3.6 ± 0.3 ab	4.8 ± 0.2 a	6.1 ± 0.2 ab	6.1 ± 0.3 ab	5.8 ± 0.2 ab
SinC15	3.1 ± 0.5 b	4.4 ± 0.2 b	5.9 ± 0.4 b	5.8 ± 0.6 b	5.6 ± 0.7 b
Regime 2 (pH 4.5, EC 3.0 dS m^{-1})					
Stone wool	3.9 ± 0.5 ns	5.0 ± 0.2 a	6.6 ± 0.2 a	6.2 ± 0.6 ab	6.4 ± 0.3 a
Coir	3.8 ± 0.3	5.2 ± 0.7 a	6.6 ± 0.4 a	6.5 ± 0.3 a	6.1 ± 0.3 ab
GigS5	3.7 ± 0.5	4.8 ± 0.3 ab	6.0 ± 0.2 b	6.2 ± 0.2 ab	5.8 ± 0.3 b
GigC15	3.8 ± 0.2	4.6 ± 0.4 ab	5.9 ± 0.3 b	5.9 ± 0.5 b	5.6 ± 0.2 b
RobC15	3.7 ± 0.5	4.8 ± 0.3 ab	6.0 ± 0.3 b	6.2 ± 0.3 ab	5.7 ± 0.3 b
SinC15	3.4 ± 0.3	4.4 ± 0.3 b	5.9 ± 0.4 b	6.0 ± 0.5 ab	5.6 ± 0.4 b
Stem length (cm)					
Regime 1 (pH 5.5, EC 2.6 dS m^{-1})					
Stone wool	56.3 ± 4.7 ns	127.4 ± 8.9 ns	368.6 ± 15.9 ns	464.4 ± 18.6 ns	608.7 ± 26.9 ns
Coir	60.4 ± 4.0	133.7 ± 7.9	366.4 ± 11.3	454.1 ± 39.4	607.1 ± 15.0
GigS5	60.1 ± 3.0	130.4 ± 6.9	354.6 ± 16.8	451.1 ± 22.8	595.0 ± 26.5
GigC15	59.4 ± 4.2	130.9 ± 8.8	353.8 ± 13.5	450.8 ± 14.1	589.8 ± 19.5
RobC15	57.9 ± 3.7	129.3 ± 6.5	344.7 ± 26.3	448.2 ± 12.8	585.0 ± 20.8
SinC15	58.3 ± 6.5	138.9 ± 8.6	370.4 ± 17.1	468.1 ± 9.9	613.7 ± 11.8
Regime 2 (pH 4.5, EC 3.0 dS m^{-1})					
Stone wool	60.8 ± 4.8 ns	129.9 ± 8.4 ns	353.2 ± 13.1 ns	449.3 ± 13.4 ns	584.3 ± 16.6 ns
Coir	59.3 ± 3.9	129.9 ± 10.0	352.5 ± 15.3	444.1 ± 12.5	581.0 ± 21.8
GigS5	61.1 ± 7.6	132.8 ± 12.2	357.8 ± 13.0	448.8 ± 13.8	584.0 ± 13.5
GigC15	64.1 ± 2.9	134.3 ± 8.6	359.2 ± 13.0	442.9 ± 19.2	585.2 ± 14.9
RobC15	60.6 ± 7.6	133.9 ± 10.8	361.4 ± 12.7	452.0 ± 11.2	591.2 ± 17.4
SinC15	59.1 ± 3.4	135.2 ± 8.7	367.1 ± 15.3	462.9 ± 13.8	603.1 ± 21.5

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, $n = 8$), ns: non-significant. DAT: days after transplanting. Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Table 3.7 Biomass and leaf area of single pruned leaf

Substrate	Fresh biomass and leaf area of single pruned leaf at				
	34 DAT	47 DAT	61 DAT	76 DAT	106 DAT
Fresh biomass of pruned leaf (g)					
Regime 1 (pH 5.5, EC 2.6 dS m^{-1})					
Stone wool	9.9 ± 1.5 ab	35.5 ± 5.5 ab	63.9 ± 7.6 a	71.7 ± 17.6 a	45.2 ± 9.8 ns
Coir	11.2 ± 0.8 a	38.2 ± 5.7 a	64.7 ± 5.9 a	56.1 ± 16.4 ab	37.9 ± 12.8
GigS5	9.9 ± 1.0 ab	31.3 ± 3.0 b	56.4 ± 7.2 ab	54.4 ± 16.9 ab	38.2 ± 11.0
GigC15	9.2 ± 0.9 b	29.7 ± 3.2 b	48.8 ± 9.3 b	52.6 ± 12.2 ab	39.5 ± 12.8
RobC15	9.7 ± 1.2 ab	30.8 ± 3.4 b	52.7 ± 5.2 b	46.5 ± 14.9 b	38.0 ± 12.4
SinC15	10.9 ± 1.5 ab	31.5 ± 2.1 b	46.8 ± 7.1 b	48.5 ± 15.9 ab	37.9 ± 9.1
Regime 2 (pH 4.5, EC 3.0 dS m^{-1})					
Stone wool	10.5 ± 1.4 ns	35.1 ± 3.8 ab	60.2 ± 5.6 ab	55.2 ± 15.2 ab	41.0 ± 13.2 ab
Coir	10.1 ± 2.3	37.0 ± 8.1 a	63.6 ± 9.0 a	61.4 ± 20.5 a	44.6 ± 13.2 a
GigS5	10.3 ± 1.2	31.7 ± 3.2 ab	50.4 ± 6.0 c	43.3 ± 10.3 ab	30.8 ± 9.8 ab
GigC15	9.8 ± 1.6	30.9 ± 3.5 ab	48.0 ± 6.3 c	36.6 ± 6.1 b	26.8 ± 4.0 b
RobC15	9.3 ± 1.1	29.8 ± 2.3 b	51.8 ± 4.5 bc	49.6 ± 15.1 ab	35.2 ± 10.5 ab
SinC15	9.4 ± 1.1	29.9 ± 2.4 b	48.4 ± 2.9 c	52.1 ± 12.1 ab	34.5 ± 9.7 ab

(continue next page)

Substrate	Fresh biomass and leaf area of single pruned leaf at				
	34 DAT	47 DAT	61 DAT	76 DAT	106 DAT
Leaf area of pruned leaf (dm^2)					
Regime 1 (pH 5.5, EC 2.6 dS m^{-1})					
Stone wool	n.d.	6.33 ± 1.00 ns	14.86 ± 2.40 abc	n.d.	10.31 ± 1.70 ns
Coir		6.77 ± 1.20	17.49 ± 1.10 a		8.81 ± 2.30
GigS5		6.04 ± 0.50	14.91 ± 2.10 abc		8.48 ± 2.00
GigC15		5.54 ± 0.80	14.32 ± 2.00 bc		9.47 ± 3.10
RobC15		6.01 ± 1.00	14.96 ± 2.50 ab		8.11 ± 2.40
SinC15		6.06 ± 0.30	11.95 ± 1.70 c		9.08 ± 1.30
Regime 2 (pH 4.5, EC 3.0 dS m^{-1})					
Stone wool	n.d.	6.53 ± 0.91 ns	16.11 ± 2.17 a	n.d.	9.71 ± 2.79 ns
Coir		6.50 ± 1.82	15.81 ± 1.77 a		9.99 ± 2.82
GigS5		6.28 ± 0.78	14.92 ± 1.77 ab		7.53 ± 2.23
GigC15		6.30 ± 1.16	14.24 ± 1.77 ab		7.56 ± 2.39
RobC15		5.84 ± 0.79	14.06 ± 1.61 ab		7.77 ± 2.66
SinC15		6.10 ± 0.78	12.51 ± 1.34 b		8.49 ± 2.03

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 8), ns: non-significant. n.d. not determined. DAT: days after transplanting. Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* and *M. sinensis* hammermilled through a 15-mm screen (chips), respectively.

Table 3.8 SPAD values of tomato plants over the course of cultivation

Substrate	Regime	SPAD values at			
		36 DAT	57 DAT	105 DAT	133 DAT
Stone wool	Regime 1	44.0 ± 3.0 b	40.9 ± 2.5 ns	49.0 ± 2.3 ns	47.5 ± 3.1 ns
Coir		42.7 ± 2.9 b	40.2 ± 2.4	46.9 ± 3.6	48.3 ± 2.1
GigS5		44.9 ± 2.9 ab	42.6 ± 2.2	48.1 ± 2.3	48.3 ± 1.6
GigC15		43.1 ± 1.5 b	40.3 ± 1.4	47.2 ± 1.1	48.5 ± 2.1
RobC15		45.0 ± 1.6 ab	42.4 ± 1.8	48.5 ± 1.5	48.3 ± 2.0
SinC15		48.4 ± 4.3 a	43.2 ± 3.3	48.8 ± 1.3	50.0 ± 3.7
Stone wool	Regime 2	43.3 ± 1.4 ns	39.9 ± 1.9 ns	49.0 ± 1.3 ns	48.8 ± 2.0 ns
Coir		43.1 ± 2.2	39.7 ± 3.2	46.8 ± 1.9	48.0 ± 2.2
GigS5		44.7 ± 2.0	42.2 ± 2.5	47.0 ± 2.0	49.6 ± 1.6
GigC15		44.3 ± 3.4	40.7 ± 3.6	46.8 ± 1.6	49.0 ± 2.3
RobC15		44.3 ± 2.5	40.8 ± 2.8	46.8 ± 2.6	49.4 ± 1.6
SinC15		43.4 ± 2.1	40.7 ± 2.2	46.2 ± 2.2	49.3 ± 2.0

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 8), ns: non-significant. DAT: days after transplanting. Regime 1: pH 5.5, EC 2.6 dS m^{-1}; regime 2: pH 4.5, EC 3.0 dS m^{-1}. Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Fruit yield

Tomato fruit yield was different among the substrates (Figure 3.5). Up to truss 20, in regime 1, plants in coir had the highest fruit yield (10.0 kg $plant^{-1}$). Plants in GigC15 showed higher yield than those in stone wool (8.5 vs. 7.1 kg $plant^{-1}$), while plants in other miscanthus substrates produced similar yield to those in stone wool (7.2–8.1 kg $plant^{-1}$). In regime 2, plants in all miscanthus substrates produced higher fruit yield than those in stone wool (7.1–7.3 vs. 5.1 kg $plant^{-1}$). In both regimes, from truss 4 and truss 5 onwards, yield difference occurred between coir and stone wool, miscanthus substrates. From truss 9 onwards, yield difference among miscanthus substrates and stone wool started.

Yield difference mainly caused by BER incidence (Figure 3.6). Plants grown in coir had the lowest number of BER fruit in both regimes (17 and 23 BER fruits per plant over 20 trusses in regime 1 and regime 2, respectively). Plants in stone wool had highest number of BER fruit in both regimes (48 and 66 BER fruits per plant in regime 1 and regime 2, respectively). Among miscanthus substrates, in regime 2, plants in all miscanthus substrates had same BER fruit number (37–42 BER fruits per plant), higher than coir and lower than stone wool. In regime 1, genotype effect showed that plants in GigC15 and SinC15 had lower BER than plants in stone wool, while those in GigS5 and RobC15 showed similar BER fruit number to those in stone wool.

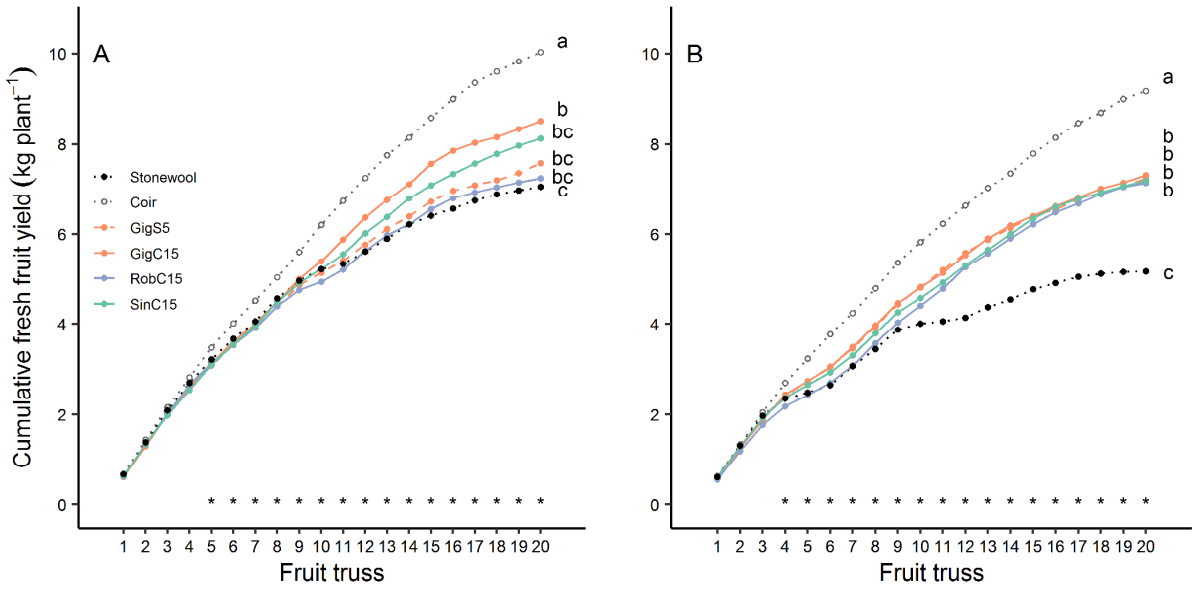

Figure 3.5 Cumulative fresh fruit yield of tomatoes grown in different substrates under (A) input regime 1 (pH 5.5, EC 2.6 dS m^{-1}) and (B) input regime 2 (pH 4.5, EC 3.0 dS m^{-1}).
The (*) at each truss and lower case letters at truss 20 indicate statistically significant differences on cumulative means up to corresponding truss among substrates (ANOVA, Tukey's HSD, $p \leq 0.05$, $n = 12$). Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

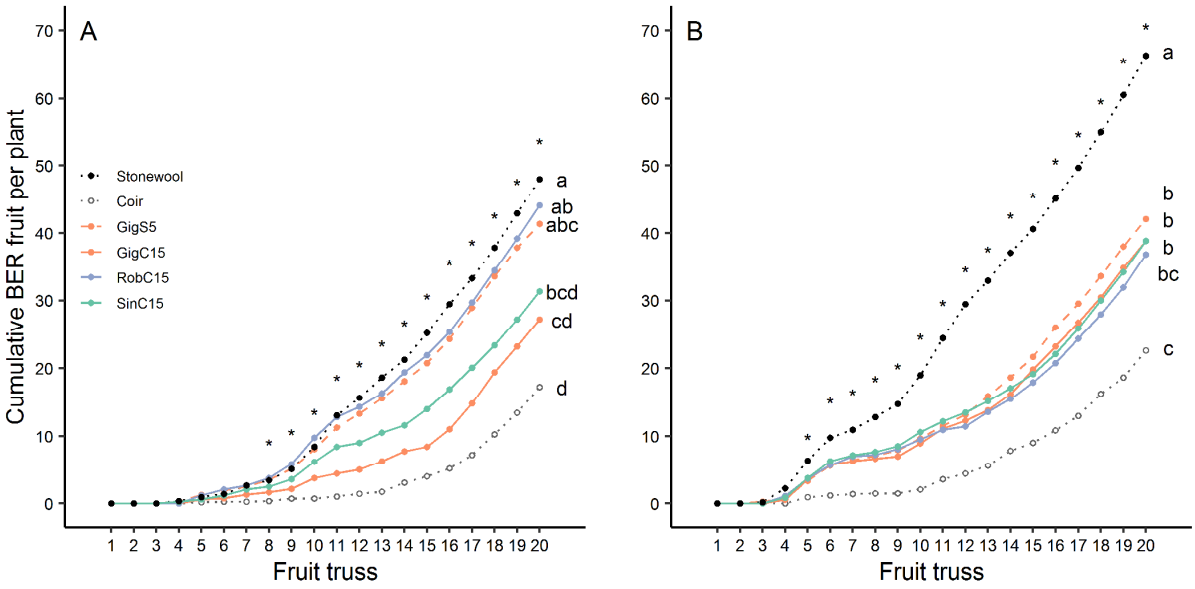

Figure 3.6 Cumulative blossom-end rot (BER) fruits per plant of tomatoes grown in different substrates under (A) input regime 1 (pH 5.5, EC 2.6 dS m^{-1}) and (B) input regime 2 (pH 4.5, EC 3.0 dS m^{-1}). The (*) at each truss and lower case letters at truss 20 indicate statistically significant differences on cumulative means up to corresponding truss among substrates (ANOVA, Tukey's HSD, $p \leq 0.05$, n = 12). Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Fruit quality

Fruits from plants grown in tested substrates had total soluble solids in the normal range (Table 3.9). In regime 1, tomatoes grown in miscanthus substrates produced similar fruit quality in terms of total soluble solids, fruit weight and fruit dry matter, except the difference in fruit dry matter (Δ = 0.6-0.9%) between GigS5 and SinC15. In regime 2, fruit weight and fruit dry matter were similar among the substrates. Tomatoes in SinC15 had higher Brix value (Δ = 0.5-0.6 °Brix) than those in coir in truss 1 in both regimes.

Table 3.9 Total soluble solids, fruit weight and fruit dry matter of single tomato fruit in selected trusses

Substrate	Total soluble solids (°Brix)		Single fruit weight (g)		Percentage fruit dry matter (%)	
	Truss 1	Truss 7	Truss 1	Truss 7	Truss 1	Truss 7
Regime 1			ns.	ns.		
Stone wool	4.5 ± 0.1 ab	5.1 ± 0.3 a	118.2 ± 8.9	90.2 ± 10.7	5.4 ± 0.1 b	4.8 ± 0.3 b
Coir	4.3 ± 0.2 b	4.4 ± 0.3 b	112.1 ± 19.3	108.6 ± 28.6	5.4 ± 0.2 b	4.8 ± 0.2 b
GigS5	4.5 ± 0.5 ab	4.4 ± 0.6 b	117.1 ± 25.7	80.5 ± 14.3	5.4 ± 0.4 b	5.8 ± 0.6 a
GigC15	4.5 ± 0.1 ab	4.7 ± 0.3 ab	128.8 ± 14.3	91.1 ± 13.6	5.6 ± 0.1 ab	5.5 ± 0.6 ab
RobC15	4.7 ± 0.3 ab	4.6 ± 0.5 ab	113.2 ± 18.9	82.0 ± 7.2	5.7 ± 0.1 ab	5.4 ± 0.7 ab
SinC15	4.8 ± 0.2 a	4.8 ± 0.4 ab	114.9 ± 21.7	93.1 ± 22.9	6.0 ± 0.2 a	4.7 ± 0.3 b
Regime 2			ns.	ns.	ns.	ns.
Stone wool	4.9 ± 0.2 ab	4.8 ± 0.3 ab	109.5 ± 12.3	89.1 ± 21.8	5.6 ± 0.1	5.0 ± 0.4
Coir	4.6 ± 0.2 b	4.7 ± 0.5 b	112.2 ± 20.3	85.0 ± 17.7	5.8 ± 0.2	5.0 ± 0.4

GigS5	4.8 ± 0.2 b	5.0 ± 0.3 ab	105.3 ± 3.3	81.5 ± 14.1	5.9 ± 0.3	5.6 ± 0.5
GigC15	4.6 ± 0.2 b	4.8 ± 0.4 ab	104.0 ± 32.4	83.1 ± 19.2	5.9 ± 0.2	5.3 ± 0.5
RobC15	4.9 ± 0.2 ab	5.1 ± 0.2 ab	112.8 ± 14.4	90.8 ± 9.6	5.7 ± 0.4	5.3 ± 0.6
SinC15	5.2 ± 0.2 a	5.4 ± 0.2 a	113.3 ± 10.5	74.0 ± 6.5	6.0 ± 0.2	5.0 ± 0.3

Different lower case letters indicate statistically significant differences among substrates in each column (Tukey's HSD, $p \leq 0.05$, n = 6 for truss 1, n = 8 for truss 7), ns.: non-significant. Regime 1: pH 5.5, EC 2.6 dS m^{-1}; regime 2: pH 4.5, EC 3.0 dS m^{-1}. Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Combustion quality of the substrates

Calorific values of the new feedstock and the substrates after they have been used as soilless substrates were comparable (Table 3.10). Although used substrates showed slightly lower calorific values (the difference less than 1 MJ kg^{-1}), the absolute values were in the reported range of heating value for miscanthus ranging from 17 to 20 MJ kg^{-1} (Brosse et al., 2012). As raw materials, SinC15 and RobC15 had higher ash content than GigC15 and GigS5, which could be explained by the higher leaf proportion in harvested biomass of the genotypes *M. sinensis* 21 and *M. 'Robustus'* 24 (Table 3.3). Compared to new materials, ash content in used miscanthus substrates increased with a factor from two/three (GigS5, RobC15 and SinC15) to five (GigC15). For both regimes, the used GigS5 had highest calorific values, but lowest ash content.

Table 3.10 Combustion quality of miscanthus substrates before and after being used as soilless substrates for tomato cultivation

Parameter	Substrate	New material	Used material	
			Regime 1	Regime 2
Calorific value (MJ kg^{-1})	GigS5	18.5 ± 0.4 ns.	18.3 ± 0.2 a	18.6 ± 0.1 a
	GigC15	18.4 ± 0.3	17.8 ± 0.1 ab	17.9 ± 0.4 ab
	RobC15	18.1 ± 0.5	18.0 ± 0.4 ab	17.3 ± 1.0 b
	SinC15	17.9 ± 0.4	17.4 ± 0.6 b	17.3 ± 0.7 ab
Ash content (%)	GigS5	3.2 ± 0.6 b	8.4 ± 1.6 b	7.5 ± 0.6 b
	GigC15	2.5 ± 0.8 b	11.6 ± 0.9 ab	10.6 ± 1.4 a
	RobC15	4.6 ± 0.3 a	9.4 ± 1.0 b	12.1 ± 1.0 a
	SinC15	4.5 ± 0.5 a	12.9 ± 1.1 a	12.5 ± 0.7 a

Different lower case letters indicate statistically significant differences among substrates in each column (Tukey's HSD, $p \leq 0.05$, n = 4), ns.: non-significant. Regime 1: pH 5.5, EC 2.6 dS m^{-1}; regime 2: pH 4.5, EC 3.0 dS m^{-1}. Stone wool: commercial Rockwool Grodan Grotop master. Coir: commercial Jiffy High Yield Growbag double-layered. GigS5: *M.* x *giganteus* processed with a mechanical fraying facility through a 5-mm screen (shreds). GigC15, RobC15, SinC15: *M.* x *giganteus*, *M.* '*Robustus'* 24 and *M. sinensis* 21 hammermilled through a 15-mm screen (chips), respectively.

Experiment 2019: Effects of substrate amendment with $Ca(NO_3)_2$ and elemental sulfur on tomato growth

pH, EC and NO_3^- in root-zone solution

pH evolution was similar to the pattern observed in the experiment 2018: pH increased to highest values around 46 DAT, then decreased to its lowest values around 111 DAT (Figure 3.7). Miscanthus substrates showed highest pH values around pH 7. Miscanthus amended with elemental sulfur did not

reduce substrate pH. Miscanthus amended with $Ca(NO_3)_2$ had highest pH values at the early stage, and then decreased to similar values of other miscanthus substrates.

In the first few weeks (until 21 DAT), EC values in miscanthus substrates amended with $Ca(NO_3)_2$ were extremely high (Figure 3.7), due to the remaining $Ca(NO_3)_2$ solution after pre-wetting step. After 21 DAT, substrates showed no difference in root-zone EC. At the early stage (until 21 DAT), nitrate concentration was not different among substrates, except the substrates with $Ca(NO_3)_2$ had higher nitrate concentration. After that, nitrate concentration in stone wool and coir showed higher values than in miscanthus. No difference in nitrate concentration among miscanthus substrates were found.

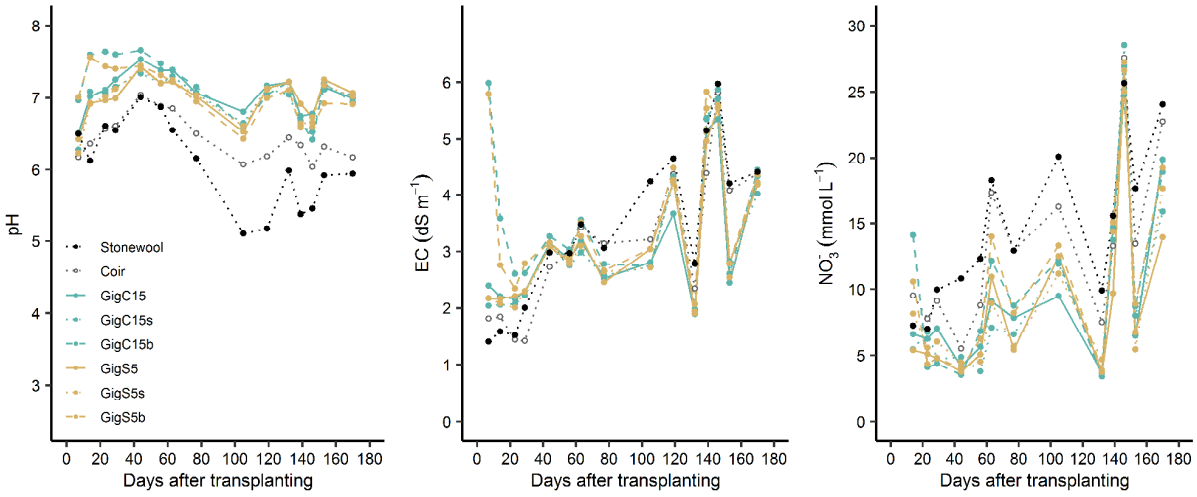

Figure 3.7 pH, EC and nitrate concentration in root-zone solution over the course of cultivation (n = 4). GigC15, GigC15s, GigC15b: hammermilled *M.* x *giganteus* processed through a 15-mm screen with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively. GigS5, GigS5s, GigS5b: shredded *M.* x *giganteus* processed through a 5-mm screen size with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively.

Early vegetation growth

Tomato plants grown in all substrates had their first anthesis in the period from 20 to 29 DAT. The harvest of the first truss occurred from 97 to 104 DAT. Within the first month after transplanting, vegetative growth of tomatoes expressed as sucker biomass and stem growth was comparable among the substrates (Table 3.11). From the second month after transplanting, biomass of pruned leaves from the first three pruning times indicated that plants in miscanthus substrates had similar growth to those in stone wool, but lower than those in coir (Table 3.12).

Table 3.11 Biomass of pruned suckers from the first pruning, stem length and stem circumference of tomatoes

Substrate	Sucker biomass at 15 DAT (g)	Stem length at 31 DAT(cm)	Stem circumference at 31 DAT (cm)
Stone wool	0.96 ± 0.75 ns	105.1 ± 7.1 ns	5.3 ± 0.4 ab
Coir	1.45 ± 2.03	108.7 ± 5.7	5.4 ± 0.4 a
GigC15	1.38 ± 1.64	106.0 ± 6.8	5.1 ± 0.5 ab
GigC15s	0.78 ± 0.43	106.8 ± 6.8	5.0 ± 0.3 ab
GigC15b	1.09 ± 0.77	107.3 ± 6.8	5.2 ± 0.3 ab
GigS5	0.91 ± 0.68	107.8 ± 5.9	4.9 ± 0.3 b
GigS5s	0.92 ± 0.93	108.8 ± 7.3	5.0 ± 0.3 ab
GigS5b	0.99 ± 0.74	106.7 ± 4.8	5.0 ± 0.3 ab

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 12), ns.: non-significant. DAT: days after transplanting. GigC15, GigC15s, GigC15b: hammermilled *M.* x *giganteus* processed through a 15-mm screen with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively. GigS5, GigS5s, GigS5b: shredded *M.* x *giganteus* processed through a 5-mm screen size with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively.

Table 3.12 Leaf area and fresh weight of single pruned leaf

Substrate	Leaf area (dm^2) at			Fresh weight (g) at		
	41 DAT	51 DAT	58 DAT	41 DAT	51 DAT	58 DAT
	ns.	ns.	ns.			
Stone wool	4.59 ± 0.33	10.93 ± 0.71	12.33 ± 1.10	18.5 ± 2.2 ab	46.6 ± 3.2 b	64.3 ± 8.8 b
Coir	4.53 ± 0.43	10.77 ± 3.38	15.09 ± 1.25	21.0 ± 1.8 a	53.5 ± 5.1 a	74.7 ± 6.9 a
GigC15	4.41 ± 0.52	10.39 ± 0.51	12.5 ± 1.10	18.0 ± 2.2 b	43.6 ± 5.1 b	57.8 ± 5.9 b
GigC15s	4.23 ± 0.31	10.36 ± 0.62	13.39 ± 2.64	17.8 ± 1.8 b	43.5 ± 3.5 b	62.5 ± 8.1 b
GigC15b	4.48 ± 0.54	11.72 ± 1.38	13.15 ± 0.99	17.6 ± 2.6 b	45.0 ± 5.8 b	59.3 ± 7.6 b
GigS5	4.48 ± 0.46	10.48 ± 0.77	12.71 ± 2.52	16.8 ± 2.3 b	41.8 ± 4.4 b	57.7 ± 8.2 b
GigS5s	4.47 ± 0.28	10.96 ± 0.92	13.22 ± 1.73	17.3 ± 2.0 b	42.8 ± 4.7 b	59.8 ± 5.5 b
GigS5b	4.26 ± 0.61	10.46 ± 0.81	12.75 ± 0.87	16.3 ± 2.2 b	40.9 ± 4.2 b	60.4 ± 5.1 b

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 5 for leaf area, n = 10 for leaf weight), ns.: non-significant. DAT: days after transplanting. GigC15, GigC15s, GigC15b: hammermilled *M.* x *giganteus* processed through a 15-mm screen with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively. GigS5, GigS5s, GigS5b: shredded *M.* x *giganteus* processed through a 5-mm screen size with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively.

Yield and fruit quality

Plants in all miscanthus substrates produced similar fruit yield to those in stone wool (8.4-9.4 kg $plant^{-1}$), but lower than those in coir (10.8 kg $plant^{-1}$) (Figure 3.8). The yield pattern was reversible to the BER incidence. BER occurred from truss 8 and increased rapidly from truss 12 upwards. Plants in coir had the lowest BER fruit number (26 fruits), where those in miscanthus and stone wool had higher BER fruit number (35-40 fruits). Fruit quality parameters were similar among all tested substrates (Table 3.13). No effect of substrate amendment or substrate processing type was observed in measured yield parameters.

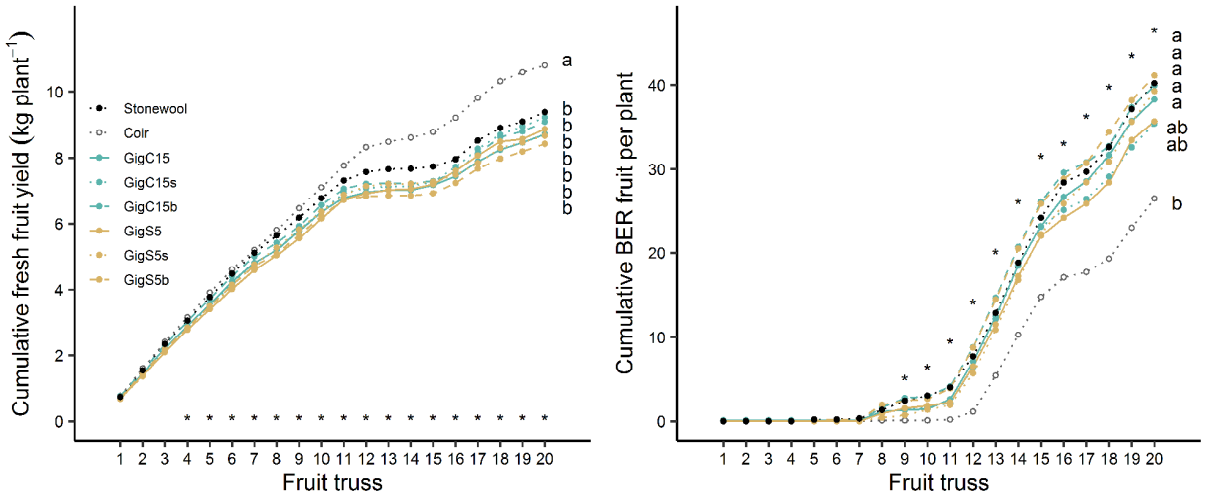

Figure 3.8 Cumulative fresh fruit yield and blossom-end rot (BER) fruit number per plant of tomatoes. The (*) at each truss and different lower case letters at truss 20 indicate statistically significant differences on cumulative means up to corresponding truss among substrates. GigC15, GigC15s, GigC15b: hammermilled *M.* x *giganteus* processed through a 15-mm screen with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively. GigS5, GigS5s, GigS5b: shredded *M.* x *giganteus* processed through a 5-mm screen size with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively (n = 20).

Table 3.13 Total soluble solids, fruit weight and fruit dry matter of single tomato fruit in selected trusses

Substrate	Total soluble solids (°Brix)		Single fruit weight (g)		Percentage fruit dry matter (%)	
	Truss 1	Truss 5	Truss 1	Truss 5	Truss 1	Truss 5
	ns.	ns.	ns.	ns.	ns.	ns.
Stone wool	3.8 ± 0.3	4.9 ± 0.2	111.9 ± 15.6	125.7 ± 11.5	4.4 ± 0.2	5.2 ± 0.3
Coir	4.1 ± 0.2	4.7 ± 0.6	121.6 ± 12.8	134.1 ± 20.2	4.7 ± 0.2	5.6 ± 0.4
GigC15	4.2 ± 0.3	4.5 ± 0.3	126.9 ± 35.7	130.3 ± 17.1	4.8 ± 0.3	5.4 ± 0.2
GigC15s	4.3 ± 0.5	4.9 ± 0.4	121.4 ± 22.0	121.0 ± 15.8	4.9 ± 0.4	5.4 ± 0.3
GigC15b	4.0 ± 0.4	4.7 ± 0.4	125.4 ± 17.0	137.5 ± 12.5	4.8 ± 0.3	5.5 ± 0.4
GigS5	4.1 ± 0.3	4.8 ± 0.4	116.8 ± 21.2	132.8 ± 33.2	4.6 ± 0.3	5.6 ± 0.3
GigS5s	4.2 ± 0.3	5.1 ± 0.6	120.0 ± 19.0	105.3 ± 21.7	4.8 ± 0.4	5.9 ± 0.8
GigS5b	4.2 ± 0.3	4.9 ± 0.1	113.2 ± 25.7	123.8 ± 22.1	4.8 ± 0.3	5.2 ± 0.4

Different lower case letters indicate statistically significant differences in means among substrates at each parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 6). GigC15, GigC15s, GigC15b: hammermilled *M.* x *giganteus* processed through a 15-mm screen with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively. GigS5, GigS5s, GigS5b: shredded *M.* x *giganteus* processed through a 5-mm screen size with no further treatment, amended with elemental sulfur or amended with $Ca(NO_3)_2$, respectively.

4. Discussions

Effects of miscanthus genotypes, miscanthus processing and substrate amendment with elemental sulfur on pH in root-zone solution

In both experiments, from transplanting to the end of anthesis period of the first truss (around 5-6 weeks after transplanting), pH in root-zone solution of all substrates increased gradually to highest values, then reduced to lowest values towards the early stage of harvest period (Figure 3.2, 3.7). Xiong et al., 2018 observed similar trend of pH change in soilless tomatoes grown in stone wool and coir, that highest pH occurred at early growing period (around 4–5 weeks after transplanting), then pH reduced to lowest value around 14 weeks after transplanting and maintained stable towards the end of cultivation. Guo-jing et al., 2002 also reported same pattern of pH change in drained solution of cherry tomatoes

with highest pH occurred around 6 weeks after transplanting. This pattern of pH change could be explained as a response to plant growth (Sonneveld & Voogt, 2009). In response to strong vegetative growth, plant roots uptake much NO_3^- which resulted in pH increase (Grodan, n.d.). With a greater uptake of cations such as K^+ in response to a high fruit load, the pH will decrease.

While the pH change over the course of cultivation depended on plant growth, the fluctuation degree was substrate-dependent. As inert material, pH in stone wool fluctuated at the greatest extent compared to organic materials as coir and miscanthus (Figure 3.2, 3.7). Coir had initial pH of 5.2 and fluctuated around the range of 5.5–6.0 at cultivation. Miscanthus substrates had acidic pH 6 (except SinC15 with pH 6.9), then their pH values increased and maintain around 7.0–7.5 at cultivation. Guo-jing et al., 2002 observed that miscanthus pH was 6, then increased to 8 when being used in a mixture of 80% miscanthus and 20% wood fiber to grow tomatoes. The authors suggested that high bicarbonate (HCO_3^-) content (1.9 mmol L^{-1}) in miscanthus substrate might cause pH rise. They observed that during growing period, HCO_3^- concentration in drained solution paralleled pH reduction. In our study, due to limited budget, there was no data on chemical composition in root-zone solution to figure out which chemical factors might cause the shift in pH. However, the pH change when adding nutrient solutions with pH 2, 5 and 9 suggested that miscanthus substrates tend to buffer their pH to 7 (Figure 3.1). Substrate from *M. sinensis* (SinC15) showed higher pH also increased pH to higher value. In tomato cultivation, genotype treatment, however, showed no effect on pH in root-zone solution.

Processing miscanthus into smaller particle size might reduce substrate pH. However, the effect of particle size on substrate pH could only observed in the experiment in 2018 in input regime2 with pH 4.5 and EC 3.0 dS m^{-1}. In regime 1 with pH 5.5 and in the experiment in 2019, no difference in substrate pH between GigC15 and GigS5 was found (Figure 3.2, 3.7).

In the experiment in 2019, elemental sulfur treatment did not reduce pH in miscanthus substrates (Figure 3.7). Elemental sulfur has been shown to successfully reduce substrate pH of switchgrass in pots (Altland & Krause, 2010). In a preliminary test, pH reduction in miscanthus substrate amended with elemental sulfur was observed in pots (data not shown). However, in growbags, elemental sulfur did not show its effect on pH reduction. This could be explained that the semi-flooding condition inside the growbags might inhibit the oxidation of sulfur into sulfuric acid by the bacteria, mainly *Thiobacillus*. Instead, elemental sulfur was converted to hydrogen sulfide by anaerobic bacteria. This could be confirmed by the rotten egg smell at the early weeks inside the greenhouse. The smell disappeared later. Therefore, pH reduction with elemental sulfur could work in pots, but not in growbags.

Effects of miscanthus genotypes, miscanthus processing types and substrate amendment with $Ca(NO_3)_2$ on the EC and NO_3^- concentration in root-zone solution

Over the course of cultivation, EC values in root-zone solution were similar among miscanthus substrates (Figure 3.2, 3.7). No genotype effect or substrate amendment was found, except the high EC values at the beginning in miscanthus substrates amended with $Ca(NO_3)_2$ (Figure 3.7). The high

concentration of Ca^{2+} and NO_3^- caused the rise in EC. After few weeks, the ions were washed out of the growbag, then the EC values in $Ca(NO_3)_2$ reduced to the same values of other miscanthus substrates. Lower nitrate concentration in root zone solution in miscanthus substrates indicated N immobilization. Guo-jing et al., 2002 observed lower nitrate concentration in drained solution of the mixture of 80% miscanthus and 20% wood fiber compared to PUR substrate. Both Guo-jing et al., 2002 and Xiong et al., 2018 observed the increasing nitrate concentration in drained solution of tomatoes over growing period. In this study, the nitrate in stone wool and coir increased from 5 mmol to 20 mmol with fluctuation in between (Figure 3.7).

Vegetative growth

Despite the difference in pH, EC and nitrate concentration in root-zone solution among miscanthus substrates, stone wool and coir, vegetative growth of tomato plants did not show big difference. Plants in miscanthus substrates had similar vegetative growth to those in stone wool, and lower than those in coir. Among the miscanthus genotypes, plants in SinC15 showed the lowest vegetative growth at the early growth stage only. Given the vegetative performance of tomato plants in substrates was similar between the two experiments, tomato plants might be the dominant driver controlling its vegetative growth.

Fruit yield

In both experiments, miscanthus genotype or amendment treatments showed no effect on tomato yield (Figure 3.5, 3.6, 3.8). Yield difference among tested substrates mainly caused by yield loss by BER fruits. Under reference nutrient regime for growing tomatoes in stone wool (i.e. regime 1 in the experiment 2018 and the experiment 2019), plants in miscanthus substrates had similar number of BER fruits and similar yield to those in stone wool, but showed higher BER fruit number and lower yield compared to those in coir.

Although fruit loss by BER is highly economically important and has been extensively studied, the causes of BER are still not clearly understood (Djangsou et al., 2019; Saure, 2014). Ca deficiency is often considered to be the cause of BER; however, several studies have suggested that lower Ca concentration in fruits might be a consequence of BER, rather than the cause (Franco et al., 1999; Nonami et al., 1991). Other abiotic causes for BER as environmental stress, i.e. salinity, drought, heat etc., could stimulate the production of reactive oxygen species in the plant roots then trigger cell death in fruit tissue (Saure, 2014). Although this study did not measure Ca concentration in root-zone solution or Ca concentration in plant tissue, given the similar yield performance in plants grown in stone wool and miscanthus substrates, I assume that environmental stress rather nutrient availability in root-zone solution could be the main cause for BER.

In both years, high air temperature inside the greenhouse compartment occurred during the fruit development (Supplementary, figure S3.1). High temperature expressed as maximum daily temperature

occurred earlier in the cultivation in 2018 than in 2019. Also, BER occurred in lower truss in 2018 than in 2019 (4^{th} truss vs 8^{th} truss).

Tomatoes in coir showed consistent lowest BER incidence in both experiments. The coir slab consists of two layers of coir dust and coir chunk. This design could enhance aeration in root-zone solution, and then mitigate the BER caused by the heat. The higher BER incidence in miscanthus could be explained by lower aeration in miscanthus growbags. At the start of cultivation, the height of miscanthus growbags was shorter than that of coir slabs. Also, later in the cultivation, several bags of miscanthus reduced their height (only observation, no data recorded). It was caused by substrate shrinkage, a common issue in substrates with high carbon content (Cárthaigh et al., 1997; Dannehl et al., 2015; H. Jensen et al., 2001). To improve the aeration in miscanthus growbags, I would suggest to use larger substrate particles such as the harvested materials from the field and/or increasing the height of growbags.

Combustion quality of the used substrates

The used miscanthus substrates had comparable calorific value to the new ones (Table 3.10). Lower ash content of new *M.* x *giganteus* (GigC15, GigS5) was related to a higher stem-to-leaf ratio of harvested straw in comparison to *M. 'Robustus'* and *M. sinensis* (Table 3.3), as the stems has lower ash content than the leaves (Monti et al., 2018). The increase of ash content in used miscanthus substrates could be explained by the contribution of minerals from nutrient solution and tomato roots. While ash content in the used GigS5, RobC15 and SinC15 substrates increased about two/three times, ash content in used GigC15 increased five times. I assumed that root biomass could be higher in GigC15, thus ash content increased at higher factor. This study showed that genotype (Gig, Rob, Sin) and processing (C15, S5 differing in particle size and structure) of miscanthus have an effect on combustion quality of new and used feedstock. Overall, the combustion quality of miscanthus after use as a growing medium was still high and it could be used as solid fuel in a cascading manner.

5. Conclusions

The two experiments in 2018 and 2019 showed that tomato plants in miscanthus substrates produced consistent vegetative growth and fruit yield. Although the nitrate concentration in miscanthus substrates were lower than those in stone wool, vegetative growth and fruit yield of tomato plants grown in miscanthus were comparable to that in stone wool. To reduce BER incidence in miscanthus substrates, further investigation into aeration and chemical dynamic in root zone solution in growbags should be conducted. Also miscanthus genotypes could be a promising source to improve fruit quality of tomato. To conclude, these experiments showed that in soilless tomato cultivation, miscanthus can be used to replace stone wool with a benefit of subsequent utilization as solid fuel (cascade utilization).

Supplementary

Table S3.1 Nutrient concentration of input nutrient solution for tomatoes

Nutrient		Concentration
NH_4	mmol L^{-1}	1.20
K		9.50
Ca		5.40
Mg		2.40
NO_3		16.0
H_2PO_4		1.50
SO_4		4.40
Fe	µmol L^{-1}	15.0
Mn		10.0
Zn		5.0
B		30.0
Cu		0.75
Mo		0.50
EC (dS m^{-1})		2.6
pH		5.5

Table S3.2 Practices conducted in the experiment in 2018

Date	Days after transplanting	Event
08 Jan	-	Seed sowing (into stone wool plugs)
15 Jan	-	Plug transplanting (into stone wool cubes)
07 Feb	-	Cube transplanting (into growbags)
22 Feb	15	pH change in regime 2 (pH 4.5)
26 Feb	19	1st sucker biomass sampling
27 Feb	20	Onset of the anthesis of the first truss
01 Mar	22	1st stem measurement
13 Mar	34	1st leaf pruning: leaf 1 + 2 + 3
15 Mar	36	EC change in regime 2 (EC 3.0 dS m^{-1}), 1st SPAD measurement
21 Mar	42	2nd stem measurement
26 Mar	47	2nd leaf pruning: leaf 4 + 5 + 6
09 Apr	61	3rd leaf pruning: leaf 7 + 8 + 9
12 Apr	64	2nd SPAD measurement
20 Apr	72	pH change in regime 2 (pH 5.5)
24 Apr	76	4th leaf pruning: leaf 12
08 May	90	1st fruit truss harvest
23 May	105	3rd SPAD measurement, 3rd stem measurement
24 May	106	5th leaf pruning: leaf 24
20 Jun	133	4th SPAD measurement, 4th stem measurement
01 Aug	175	5th stem measurement
24 Aug	198	Growing tip cut
14 Sep	219	Water apply in regime 2 to wash salt out
28 Sep	233	Experiment end

Table S3.3 Practices conducted in the experiment in 2019

Date	Days after transplanting	Event
07 Jan	-	Seed sowing
05 Feb	-	Transplanting (into growbags)
20 Feb	15	1st sucker biomass sampling
25 Feb	20	Onset of the anthesis of the first truss
08 Mar	31	Stem measurement
18 Mar	41	1st pruned leaf measurement
28 Mar	51	2nd pruned leaf measurement
04 Apr	58	3rd pruned leaf measurement
13 May	97	1st fruit truss harvest
30 Sep	237	Experiment end

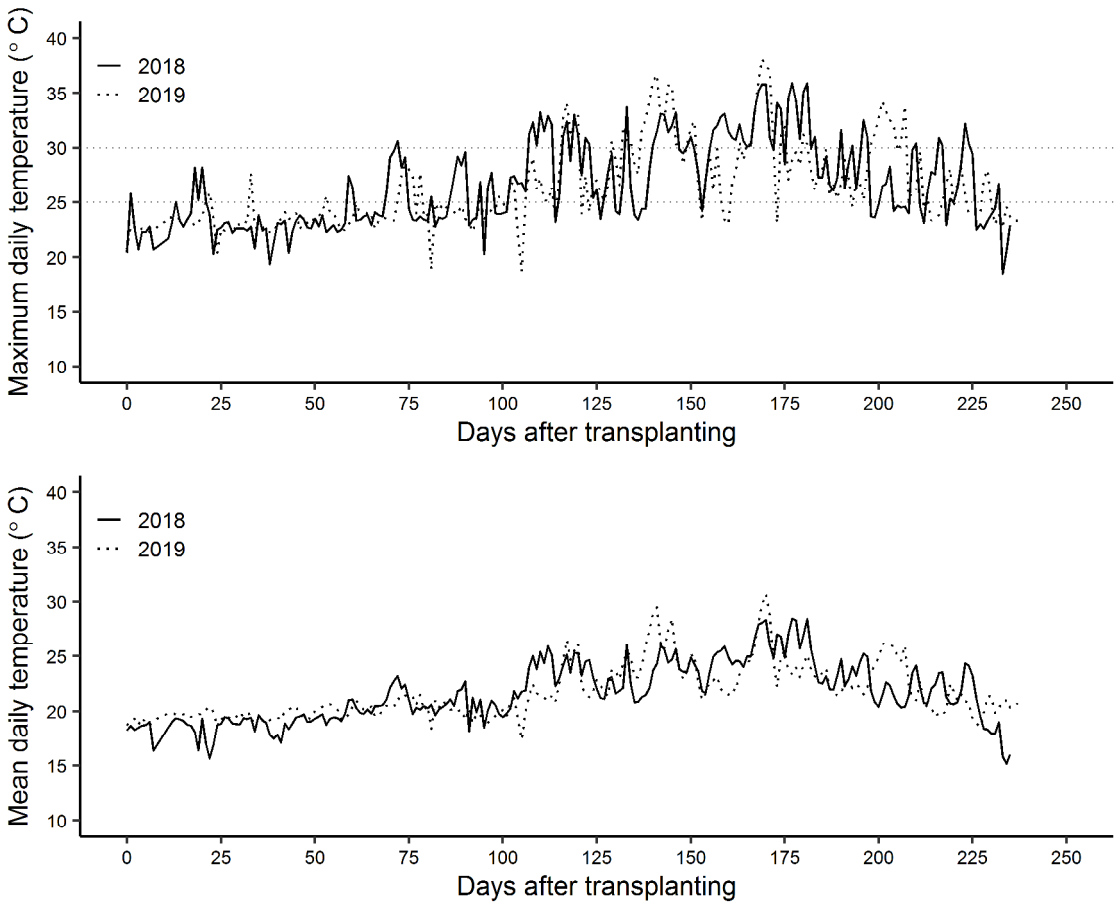

Figure S3.1 Maximum and mean daily temperature inside the greenhouse compartment in the experiment in 2018 and 2019

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Chapter 4
Primary mechanical modification to improve performance of miscanthus as stand-alone growing substrate

Abstract

Selecting proper mechanical processing provides a good start to improve performance of miscanthus substrates. We studied the effects of mechanical processing methods on substrate morphology, hydrological properties, pH and nitrogen immobilization. *Miscanthus* x *giganteus* biomass was processed into field chips (FC, forage harvester), shreds (S5, mechanical fraying machine through a 5-mm screen) and chips (C15, C10, C5 and C3, hammermill with screen size of 15, 10, 5 or 3 mm). Substrate morphology, hydrological properties, pH buffering capacity and nitrogen drawdown index (NDI) were analyzed. Processed miscanthus materials were used as propagation substrate for Chinese cabbage seedlings. Results showed that particle size distribution of miscanthus substrates formed four groups in ascending order of particle size: C3 < C5 < (C10, C15, S5) < FC. The finer miscanthus substrates had higher water holding capacity following same groupings in particle size. Hydrophobicity of processed miscanthus was low and reversible, with the increasing order of risk as C3 < C5 < C10, C15 < S5, FC. All miscanthus substrates had similar and low pH buffering capacity. NDI values were similar among miscanthus substrates (0.4), except FC (0.2). Seedlings in miscanthus substrates had similar germination rate, and lower biomass compared to those in peat and coir. The extract solution from miscanthus substrates had higher pH, lower nitrate and calcium and higher ammonium, potassium and phosphorus concentrations compared to those in peat and coir. Primary mechanical modification of miscanthus offers opportunities for different sizes of substrate materials with few changes to the physical or chemical properties tested in this work.

Keywords: growing media, substrate processing, substrate particle, particle size, particle shape, porosity, wettability, pH buffering, nitrogen immobilization, Chinese cabbage, seedling

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1. Introduction

Soilless cultivation of horticultural crops is projected to grow rapidly due to the increased production of ornamental and consumable (food) crops in soilless growing systems (Blok et al., 2021). To meet the increasing global demand for soilless substrates together with reduced availability and uncertainty of peat use in many countries across Europe, the substrate industry is searching for new alternative raw materials (Blok et al., 2021). Any suitable substrate should fulfill three requirements including reliable and consistent performance, affordability and minimal environmental impacts (Barrett et al., 2016; Gruda, 2019; Schmilewski, 2012).

Miscanthus (*Miscanthus* x *giganteus*), a perennial rhizomatous C4 grass, could be a promising renewable primary feedstock for growing substrates because of its high biomass production with low environmental impacts (low-input crop, 10–25 t dry matter ha^{-1} annually and carbon sequestration) (Clifton-Brown et al., 2004; Heaton et al., 2010; Lewandowski et al., 2000). Besides its main role as a biomass crop for biofuel, miscanthus has been investigated as soilless substrates in Europe and North America since the late 1990s. Results showed that fresh miscanthus straw could be used as substrate constituent in container substrates (20-80% volume proportion) for nursery shrubs (Altland, 2010; Altland & Locke, 2011; Bąbelewski et al., 2019; Cárthaigh et al., 1997; Frangi et al., 2012; Pancerz & Bąbelewski, 2019), tomatoes (Guo-jing et al., 2002), wood cuttings (Bąbelewski & Pancerz, 2018), strawberries (Debode et al., 2018; Vandecasteele et al., 2017) and as stand-alone substrate, i.e. sole constituent, in growbag for tomatoes and cucumbers (Kraska et al., 2018; Nguyen et al., 2021). While stand-alone miscanthus in growbags showed comparable plant yield to control substrate (stone wool) (Kraska et al., 2018; Nguyen et al., 2021), increasing proportion of miscanthus in container substrates lead to decreasing plant growth (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012). Composted miscanthus could be used as peat alternatives for nursery shrubs, but the performance of tested composts in different plant trials was inconsistent (Jensen et al., 2001a, 2001b, 2002). Reported challenges of miscanthus substrate include low water holding capacity (Altland & Locke, 2011; Cárthaigh et al., 1997), slightly high pH than the recommended range for soilless crops (Altland, 2010; Altland & Locke, 2011; Frangi et al., 2012; Guo-jing et al., 2002; Vandecasteele et al., 2018) and the risk of nitrogen (N) immobilization (Cárthaigh et al., 1997; Debode et al., 2018; Frangi et al., 2012; Guo-jing et al., 2002; Vandecasteele et al., 2018), thus substrate shrinkage for long-term cultivation (Altland & Locke, 2011; Jensen et al., 2001b). Appropriate modification strategies to overcome these challenges could determine the adoption of miscanthus in growing substrate market.

Studied modification strategies focus much on blending miscanthus with other substrate components rather than on exploring modifications on sole miscanthus substrate. The blending approach has two goals including reducing the proportion of peat or other key substrate constituents in the substrates and remedying the performance limitations of tested materials (Schmilewski, 2012). While this approach is highly effective to develop substrates with direct practical importance for horticultural production, it is limiting insight into the performance of the single feedstock. Effects of single substrate constituents on

the performance of a substrates might not be easily distinguished, particularly when our understanding of new material is limited. In order to study the performance of miscanthus substrate, we use the "stand-alone substrate" approach. By using sole substrate component and focusing on feasible modifications on material itself, we aim to develop a profile on the sole use of miscanthus as a substrate, which is useful both in developing further modifications and in formulating 100% miscanthus substrates in the future if needed.

Mechanical processing of organic biomass materials is a primary modification applied to substrate components/feedstock in order to gain desired physical properties. Substrate morphology plays an important role in determining substrate physical and hydrological properties. Studies on other organic substrates (peat, coir, bark and wood fiber) showed that finer substrate particles, i.e. particles smaller than 0.5 mm, generally increase water holding capacity and decrease air-filled porosity in formulated substrates (Abad et al., 2005; Cannavo & Michel, 2013; Caron et al., 2005; Gruda et al., 2001; Handreck, 1983; Jackson et al., 2010; Jackson & Wright, 2009). Modifying substrate morphology might also alter substrate pH and N immobilization to a certain extent. For example, fine switchgrass particles (hammermilled with 0.47 cm screen) had higher pH than coarse switchgrass (hammermilled with 1.25 to 2.5 cm screens) (Altland & Krause, 2009). Fine pine tree substrates (100% hammermilled through a 2.38 mm screen) have been shown to grow plants better than coarse pine tree substrate (hammermilled through a 4.76 mm screen) due to the higher water holding capacity (Jackson et al., 2008). Blockular shape of pine wood chips as aggregates reduced particle surface area, which might reduce the presence of microorganism, thus less N immobilization (Owen et al., 2016).

As a primary material, harvested miscanthus straw can be mechanically processed by a variety of machinery types including shredders, extruders, disc refiners or hammermills resulting in different substrate morphology like fibers, chips and shreds with various particle size distribution (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012; Kraska et al., 2018; Vandecasteele et al., 2018). Shredded, extruded and hammermilled miscanthus substrates showed different patterns of particle size distribution (Altland, 2010; Altland & Locke, 2011; Kraska et al., 2018); however, no hydrological properties of those sole substrates was investigated.

Miscanthus substrate has been reported to have a high pH in the range of 6.0–7.9 depending on the sources (Nguyen et al., 2021; Vandecasteele et al., 2017; Vandecasteele et al., 2018). Processing miscanthus straw with extruder, retruder or disc refining did not affect substrate pH, whereas steam explosion reduced substrate pH (from 6.7 to 4.0) (Vandecasteele et al., 2018). pH buffering capacity of miscanthus was thought to be low, as mixing miscanthus with a small portion of peat (20% volume) (Altland & Locke, 2011) or acid solutions (Vandecasteele et al., 2018) could bring down substrate pH. Contrarily, it has been observed that the pH in root-zone solution of tomatoes grown in miscanthus growbags was maintained around 7.0 when the pH of input fertilization was reduced to 4.5, except the shredded miscanthus decreased gradually its pH 0.5-1.0 unit over the course of cultivation compared to

hammermilled miscanthus (Nguyen et al., 2021). To our knowledge, no empirical data on pH buffering capacity of sole miscanthus substrate is available.

As a carbon (C) rich material, miscanthus substrate poses high risk for N immobilization. Increasing proportion of miscanthus in soilless substrates often reduced plant biomass (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012). Miscanthus substrates release a certain amount of water-soluble small-size C compounds which are available to microbes in substrate (Vandecasteele et al., 2018). Those microbes assimilate N in root-zone solution causing N immobilization (Vandecasteele et al., 2017). In soilless cultivation, shrubs grown in extruded miscanthus produced less biomass than those in shredded substrate (Cárthaigh et al., 1997), whereas tomatoes and cucumbers grown on extruded, shredded and hammermilled miscanthus in growbags showed similar yield to stone wool (Kraska et al., 2018). This implies that different substrate morphology and/or cultivation techniques might create favorable or less favorable conditions for microorganism growth.

Mechanical processing could be an economic primary modification to engineer raw miscanthus material. Increasing the proportion of fine particles should increase water retention of the substrate; however, it might also increase substrate pH if miscanthus substrates perform like switchgrass substrates (Altland & Krause, 2009) and increase also the amount of water-soluble C which microorganism could easily access (Vandecasteele et al., 2018). Also grinding the miscanthus to finer substrate particle size will require more energy, thus increase production costs. Selecting a proper mechanical process to engineer a sole substrate for its best performance could be a good start for further development of miscanthus as a feedstock material. In this study, our main focus was a better understanding of miscanthus performance as stand-alone substrate, focusing on the effect of substrate morphology after mechanical processing as a foundation for further modification and development.

Within this context, we sought to investigate how different mechanical processing methods influence miscanthus substrate morphology, hydrological properties, pH, and N immobilization.

2. Materials and methods

Different miscanthus substrates were produced by using a forage harvester, a mechanical fraying facility and a hammermill with different screen sizes. Then, substrate particle size and shape distribution, hydrological properties (porosity and wettability), pH buffering capacity and N immobilization without the presence of plants were analyzed. Finally, seedling growth of Chinese cabbage in substrates was investigated to quickly assess substrate performance under short-term cultivation conditions.

2.1 Substrate preparation and general substrate properties

Miscanthus (*Miscanthus* x *giganteus*) cultivated at Campus Klein-Altendorf (University of Bonn, Rheinbach, Germany) was harvested at ground level using a forage harvester (Champion C1200, Kemper, Germany) in early April 2019 after winter senescence and right before new sprout emergence. The chopped material, referred to as field-chips (FC) was stored in a protected barn until it was later processed further either with a mechanical fraying facility (Type ZF 140/B4, Eirich, Germany) with

screen size of 5 mm to produce shredded substrate (S5) or with a hammer mill (Type BHS100, Buschhoff, Germany) using different screen sizes (Table 4.1) to produce chips (C15, C10, C5 and C3). No further modification of the miscanthus materials was made. Two commercial substrates used in this study included unfertilized peat (Null-Erde, Einheitserdewerke Werkverband e.V., Germany) and unfertilized coir (RHP Legro Kokos Erdbeeren, Low Si/EC, Legro, Netherlands) (Table 4.1). Visual appearance of tested growing substrates is in the Supplementary (Figure S4.1).

Table 4.1 List of tested growing substrates

Substrate	Source	Processing method
Peat	Unfertilized peat [z]	na [y]
Coir	Unfertilized coir	
C3	*Miscanthus* x *giganteus*	Hammermill, screen 3 mm
C5		Hammermill, screen 5 mm
C10		Hammermill, screen 10 mm
C15		Hammermill, screen 15 mm
S5		Mechanical fraying facility, screen 5 mm
FC		Forage harvester

[z] Unfertilized peat consists of 70% white peat (H3–H5), 30% clay, limestone, pH 5.5–6.5
[y] na: non-applicable for commercial product

Bulk density was measured by filling fresh substrate into a cylinder (14.7 cm h x 14.1 cm i.d.) with a collar on top. The cylinder was gently dropped six times from the height of about 5 cm above a working table. After removing the collar, the substrate was levelled and weighed. Substrate was then oven-dried at 60°C for 3 days to determine dry weight. Bulk density was calculated as dry weight per volume (g cm^{-3}) (Wallach, 2019). pH (DIN EN 13037:2012-01, 2011), electrical conductivity (EC) (DIN EN 13038:2012-01, 2011) and water-soluble nutrients (DIN EN 13652:2002-01, 2002) of all substrate treatments were measured in a water-extract solution (substrate: water = 1:5, v/v). An amount of each substrate equivalent to 50 mL was added with 250 mL of demineralized water. Substrate suspension were stirred with glass stick, and then set aside at room temperature for 1 hour. It was stirred again before being filtered through a filter paper (MN616, Macherey-Nagel, Germany) to get filtered solution. pH and EC values were determine using a pH meter (pH 3000, STEP Systems GmbH, Germany) and an EC meter (FSEC20, MMM Tech Support GmbH & Co. KG, Germany), respectively. Nitrate (NO_3), ammonium (NH_4) and potassium (K) ions were measured with ion-selected electrode (MULTI ISE, Stelzner, Germany). Orthophosphate (P) was measured with the portable VIS spectrophotometer (DR 1900, Hach Lange GmbH, Germany). Calcium (Ca) was measured with Ca meter (LAQUAtwin, Horiba Scientific, Japan).

2.2 Substrate particle size and shape distribution

The profile of substrate particle size and shape was investigated with dynamic image analysis using Particle Analyzer CamSizer P4 (Microtrac Retsch GmbH, Germany) with a size class of 0.5 mm. Substrates were oven-dried at 60°C for 3 days. Mild stickiness of fine particles after ovendry was observed in peat and coir, not in miscanthus substrates. To prevent it, fresh materials of peat and coir were sieved before ovendry to separate fine and coarse particles. Dried substrates were gently mashed through a screen

in case of stickiness. Oven-dried substrates were divided into subsamples (about 25 g dry weight, 4 replicates) with the rotating Sample Divider (Microtrac Retsch GmbH, Germany). Substrates particles were added to a dosage funnel, then transported along a vibrating feeder until they fell freely into a measurement shaft where two highspeed cameras (basic and zoom cameras for large particles 300 µm–30 mm and small particles 30 µm–3 mm, respectively) would capture their images. The images would then be processed with the Camsizer software (version 6.6.5.1060) to characterize the volume-based distribution of size and shape of particle projection (Table 4.2) (Retsch Technology GmbH, n.d.).

Two main size parameters are particle width (x_{cmin}) as the shortest chord diameter and particle length (x_{Femax}) as the longest Feret diameter, which were determined out of the measured set of maximum chord diameter and maximum Feret diameter of particle projection, respectively (Table 4.2, figure 4.1). Chord diameter is the longest distance between two horizontal points of the particle contour. Feret diameter is the distance between two parallel tangents which are perpendicular to the measuring direction. The ellipsoid model was used to calculate particle volume as $V=\frac{\pi}{6}.x_{Femax}.x_{cmin}^2$. In this study, particle size distribution of substrates was described as cumulative particle size distribution, its percentile values (d10, d50, d90) and non-uniformity indicator (U3, as $\frac{d60}{d10}$). Fractions of particle size were categorized into fine (< 0.5 mm), medium (0.5–2.0 mm) and coarse particles (> 2.0) (Drzal et al., 1999). Specific surface parameter (Sv) was calculated over the whole sample as the ratio of surface area of all particles to total volume of all particles. These parameters were determined for particle width and particle length.

Particle shape distribution was monitored via aspect ratio (b/l), sphericity (SPHT), convexity (Conv) and symmetry (Symm) (Table 4.2). Values of shape parameters range between 0 and 1, with a perfect circular shape, very smooth surface or perfectly symmetrical shape having value of 1. The more particle shape deviates from circle/sphere, smooth surface or symmetrical shape, the lower the value is. As an indicator of particle elongation, aspect ratio (b/l) is calculated as the ratio of particle width (x_{cmin}) to particle length (x_{Femax}). Sphericity (SPHT) is the ratio of particle area to the perimeter of particle projection. Convexity (Conv) is the ratio of real area of a particle projection to its convex hull. The convex hull is calculated from an imaginary elastic band stretched around particle contour (Microtrac Retsch GmbH). Both sphericity and convexity indicate the surface roughness of particles (Malvern Instruments Ltd., 2015; Miller & Henderson, 2010). Symmetry measures the eccentricity of particle projection by determining the centroid of particle projection, then calculating the minimum ratio of two opposing semi axes through the centroid point. Besides the cumulative particle shape distribution of the four shape parameters, the mean values of aspect ratio, sphericity and symmetry were calculated at each size fraction (fine, medium and coarse).

Table 4.2 Volumetric parameters of particle size and shape analyzed with Particle Analyzer CamSizer

Parameter (CamSizer's parameter)	Definition [z]
Particle size	
Particle width (x_{cmin})	The shortest chord diameter of the measured set of maximum chords of a particle projection.
Particle length (x_{Femax})	The longest Feret diameter of the measured set of Feret diameter of a particle projection
Percentile values d10, d50, d90	The value of particle size when the proportion of particles with size smaller than that value is 10, 50 and 90%, respectively
Non-uniformity (U3)	$U3 = \frac{d60}{d10}$ It indicates homogeneity of particle size distribution
Specific surface (Sv)	The ratio of surface of all particles and volume of all particles
Particle shape	
Aspect ratio (b/l)	$b/l = \frac{x_{cmin}}{x_{Femax}}$ $(0 < b/l \leq 1)$ The ratio of particle width to particle length of a particle projection. It is a measurement of particle elongation (a perfect circle has b/l value of 1)
Sphericity (SPHT)	$SPHT = \frac{4\pi A}{P^2}$ $(0 < SPHT \leq 1)$ (P: measured perimeter of a particle projection, A: measured area covered by a particle projection) It is an indicator of surface roughness [y] (an ideal sphere has SPHT value of 1)
Convexity (Conv)	$Conv = \sqrt{\frac{A_{real}}{A_{convex}}}$ $(0 < Conv \leq 1)$ The ratio of the real area (A_{real}) of a particle projection to its convex hull (A_{convex}) (an imaginary elastic band stretched around the particle projection). It indicates the particle edge roughness [x] (particle with very smooth surface has Conv value of 1)
Symmetry (Symm)	$Symm = \frac{1}{2}\left[1 + \min(\frac{r_1}{r_2})\right]$ $(0 < Symm \leq 1)$ A measure of the eccentricity of the particle image by determining the centroid of particle projection, then calculating the minimum ratio of two opposing semi axes (r_1, r_2) through the centroid point (a symmetrical shape has Symm value of 1)

[z] (Retsch Technology GmbH, n.d.)
[y] (Miller & Henderson, 2010, 2011)
[x] (Malvern Instruments Ltd., 2015)

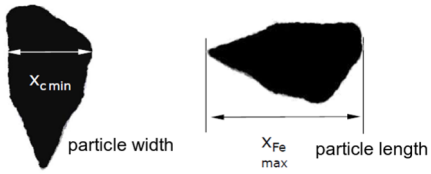


Figure 4.1 Two main particle size parameters analyzed with Particle Analyzer CamSizer: particle width (xcmin, the shortest chord diameter), particle length (xFemax, the longest Feret diameter) (adapted from (Microtrac Retsch GmbH, n.d.))

2.3 Substrate hydrological properties: porosity and wettability

Hydrological properties of substrates were characterized by porosity and wettability. Substrate porosity, i.e. total pore volume of substrate and its proportion for air and water, was determined by using a modified North Carolina State University Porometer (Fonteno & Harden, 2003). Substrate wettability describes how easily the substrate can be wetted. To assess substrate wettability, we used the hydration efficiency test developed by Fonteno *et al.* 2013 (Fields et al., 2014; Fonteno et al., 2013).

Substrate porosity: Substrates were prewetted overnight to avoid stickiness of fine particles (Supplementary, Table S4.1) A transparent acrylic column beaker served as the modified porometer (9

cm h x 6.7 cm i.d., V = 317.1 cm^3). The beaker has 5 drainage holes (5 mm diameter) at the bottom. Moist substrates were filled into beaker (with a collar on top) using a step-wise procedure (8 replicates). Substrate surface was levelled. The beakers were then placed into a level container. Water was slowly added into the container using a step-wise procedure until water level reached almost the rim of the beaker. Substrates were allowed to saturate for 30 minutes. An air-tight plastic plate and a weight were placed on beaker to create air-tight condition to prevent water leak. Beaker was then gently lifted out of the container and placed on empty cup to collect drainage water. After 30 minutes, drainage volume was recorded. Fresh weight of wet substrate was measured. Substrates were then dried in the oven at 60ºC for 3 days for dry weight. The air-filled porosity (pore volume of substrate which air occupies), water holding capacity (pore volume of substrate which water occupies) and porosity were calculated as below.

Water holding capacity: $WHC\ (\%) = 100 * (m_{substrate\ after\ drain} - m_{substrate\ after\ dry}) / V_{beaker}$

Air-filled porosity: $AFP\ (\%) = 100 * (V_{drainage} / V_{beaker})$

Porosity: $P\ (\%) = WHC + AFP$

Substrate wettability: To assess wettability, we evaluated hydration performance of substrates at different initial moisture contents (IMC) including nonwet, IMC 67%, IMC 50% and ICM 25% (w/w). Nonwet indicates the natural moisture status of miscanthus substrates and the moisture status at delivery for peat and coir, i.e. their moisture content was pre-adjusted by substrate manufacturer (Supplementary, Figure S4.2). No additional wetting was applied to nonwet substrates. Substrates at IMC 67%, 50% and 25% were prepared by wetting substrates (i.e. nonwet materials) with water to 67% (w/w), then air-drying until substrates reached their target IMC. Substrates were kept in ziplock bag to maintain their target IMC. Substrates were packed into transparent acrylic cylinders (6.5 cm i.d.) with screen at the bottom (mesh size of 1 x 1 mm) to get 230 ml of substrate. Ten successive hydration events of 230 ml water each were applied to substrate column with flowing rate of 50 ml min^{-1}. Water drainage was collected and measured after each hydration event. Water retention in substrate column was calculated as the difference between water applied (230 ml) and water drainage. After 10 events, any change in substrate heights was recorded. The water holding capacity was tested for each cylinder using the same protocol for porosity which was described previously. Hydration properties was estimated for each substrate at 4 mentioned IMC's using three criteria: (1) how fast substrate columns reached their maximum water retention within 10 events, (2) how much water absorbed after the first event compared to maximum water retention within 10 events and (3) how much maximum water retention within 10 events compared to its maximum WHC. Instead of using water holing capacity (i.e. container capacity) as reference value, we used maximum water retention within 10 applied events as it described better hydration properties of substrate from same feedstock (miscanthus) with different particle size distribution. Those criteria are presented as below parameters

Hydration speed (HS): number of hydration event applied until the substrate column reaches 90% its maximum water retention within 10 applied events.

Initial hydration efficiency (HE1): the ratio of water retention in substrate after 1st event to maximum water retention within 10 events.

Retention efficiency (RE): the ratio of maximum water retention within 10 events to WHC.

Swelling (%): the proportion of swollen volume after 10 hydration events to the initial volume of substrate

2.4 pH buffering capacity

pH buffering capacity was measured using the procedure described for compost by Costello and Sullivan (2014). First, to determine time when substrate pH become stable after acid addition, an amount of fresh C3 and S5 equivalent to 5 g of dry weight was added to a 120-ml beaker. 50 ml of three acid concentrations (HCl) were added into beakers resulted in 3 rates of 0, 0.01 and 0.1 mol H^+ kg^{-1}substrate equivalent to pH 7, pH 3 and pH 2, respectively. The beakers were closed with cap to avoid atmospheric carbon dioxide penetration. Substrate pH in the suspension was measured at 0.4, 1, 2, 3, 4, 5, 6, 7, 24 and 48 hours after acid added. The timepoint at 24 hours after acid addition was selected to determine pH buffering capacity (Supplementary, Figure S4.3). An amount of fresh substrate equivalent to 5 g of dry weight was added with 50 ml of five acid concentrations as 0, 0.01, 0.05, 0.1 and 0.3 mol H^+ kg^{-1}substrate equivalent to pH of 7.0, 3.0, 2.3, 2.0 and 1.5, respectively (5 replicates). Substrate pH was measured at 24 hours after acid addition. Linear regression was plotted between proton concentration and substrate pH. pH buffering capacity is the amount of proton needed to reduce substrate pH by one unit and is calculated as below

pH buffering capacity = (1/slope)

slope is the fitted slope of linear regression for each substrate

2.5 N immobilization

N immobilization rate was tested based on the N drawdown index (NDI) described by Handreck (1992). Substrates were incubated at 2 N rates (0 and 300 mg N L^{-1} using KNO_3 fertilizer, YaraTera™ Krista™ K Plus) for 4 days. Substrates were pre-moistened to 65% moisture content (v/v) for 8 days prior to the incubation. Substrates were then filled into commercial pots (11 cm, V of 400 ml) to the brim and placed on benches in the greenhouse. One volume of demineralized water (400 ml) was slowly poured through substrate in the pot. Charging process, i.e. adding KNO_3 solution to substrate, was done by slowly pouring 400 ml KNO_3 solution at two N rates (0 and 300 mg N L^{-}1) through substrate in the pot, waiting for 30 minutes and then adding again 400 ml KNO_3 solution. After drainage, pots weights were recorded and then covered with plastic foil with small holes to reduce evaporation. At measurement dates, demineralized water was added to bring pot weight to their initial values at the start of incubation. Substrate solution was then extracted by adding 300 ml (¾ volume of substrate volume) demineralized water, then filtering using filter paper (MN616, Macherey-Nagel, Germany). Nitrate concentration in the extract solution was measured with ion-selected electrode (MULTI ISE, Stelzner, Germany) on the

same day of extraction. NDI was calculated as the ratio of nitrate concentration in extract solution at measurement date to nitrate concentration in extract solution at the start of incubation. The smaller value of NDI (smaller than 1) indicates higher N immobilization.

2.6 Substrate performance as growing media for Chinese cabbage seedlings

Chinese cabbage (*Brassica rapa* spp. pekinensi, 'Pacifiko F1', Bejo Samen GmbH) was used to test the performance of substrates under short-term cultivation. Substrates were moistened to about 65% moisture content (v/v) right before being filled into the sowing tray (77 cells, rectangular cell 4 cm x 4 cm, 5 cm h.). One seed was hand sown into each cell. The trays were placed onto greenhouse benches with subirrigation from ebb and flow system. Since all tested substrates were unfertilized, fertigation with nutrient solution (pH 5.5, EC 2.6 dS m^{-1}, Supplementary, Table S4.2) was applied once per day (3-minute duration) beginning on the sowing date.

Germination rate was recorded daily until day 9. Seedling emergence was counted when the cotyledons came through the surface of the substrates. Mean seed emergence time (MSET) was calculated as MSET $=\sum$ n.t/$\sum$ t, with n is the number of newly emerged seedling at a time interval t. Spectral reflectance of the first leaf, i.e. the oldest leaf, was measured with the portable spectro-radiometer Polypen (Photon Systems Instruments, Czech Republic) at day 23. Based on spectral reflectance, vegetation indices as indicators for chlorophyll content were computed as below (Main et al., 2011)

Carter index = $\frac{1}{Reflectance_{550nm}}$

Datt index = $\frac{Reflectance_{672nm}}{(Reflectance_{550nm} \,.\, Reflectance_{708nm})}$

Once seedlings reached their commercial transplanting size (5-leaf stage, 24 days after sowing), we measured seedling above-ground biomass, leaf number, total leaf area for single seedling (72 seedlings each substrate). Seedlings were dried in the oven at 105°C for 2 days to get dry biomass. Substrate pH, EC and water-soluble nutrients (NO_3, NH_4, P, K and Ca) were monitored for substrate cells without seedlings (day 4, 8, 12, 16, 20 and 24) and for substrate cells with seedlings (day 9 and 17). At each sampling date, substrates from three sowing cells were sampled and loosely packed in a beaker. Demineralized water was then added slowly into the beaker to reach the saturation status. After one hour, substrate suspension was stirred with glass stick, then filtered to get extraction solution. Experiment was designed as randomized block design with four replicates, each replicate was one sowing trays with 45 seeds. One block was one bench on which 8 sowing trays of 8 tested substrates were placed.

2.7 Statistical analysis

The mean difference of tested parameters among tested substrates was conducted using one-way ANOVA. The multiple comparisons post hoc is Tukey's HSD (p-value ≤ 0.05). The R software version 4.0.2 was used.

3. Results

3.1 General substrate properties

Miscanthus substrates had different bulk density, pH, EC values and water-soluble nutrient concentration compared to peat and coir (Table 4.3). Bulk densities of miscanthus substrates were lower than peat and higher than coir. Miscanthus had lower moisture content compared to packing moisture content of commercial peat and coir (10% vs 40%-50%). Miscanthus substrates had slightly acidic pH. According to the English ADAS method (Bunt, 1988; Johnson, 1980), EC values of miscanthus were moderate to fairly high. Miscanthus substrates released a significant amount of nitrate, ammonium, potassium and calcium. Nitrate released from miscanthus substrates belonged to index 1 and 2 within the range of seven indices from low to high concentration. Having index of 2 to 3, ammonium concentration released from miscanthus was considered fairly high to young plants. Phosphorus and potassium concentration also belong to high indices.

Table 4.3 Bulk density, moisture content, pH, electrical conductivity (EC) and water-soluble of nutrients of peat, coir and processed miscanthus substrates

Substrate	Bulk density ($g\ cm^{-3}$)	Moisture content (%)	pH	EC ($dS\ m^{-1}$)	Water-soluble nutrient ($mg\ L^{-1}$ substrate)				
					NO_3	NH_4	PO_4	K	Ca
Peat	0.22 a	40.3 b	6.6 b	nd.	nd.	nd.	42.7 d	3.8 f	nd.
Coir	0.09 f	51.1 a	6.7 a	nd.	nd.	6.3 e	nd.	28.8 f	nd.
C3	0.16 b	9.7 cd	6.2 cd	0.7 a	32.5 a	117.5 a	144.3 a	1861.3 a	48.8 a
C5	0.14 c	10.1 cd	6.3 cd	0.6 b	25.0 abc	107.5 b	117.6 b	1678.8 b	33.8 ab
C10	0.13 d	10.3 cd	6.3 c	0.5 c	23.8 abc	103.8 b	101.7 b	1551.3 c	23.8 b
C15	0.12 d	9.2 d	6.3 cd	0.4 d	20.0 bc	90.0 c	67.6 c	1321.3 d	26.3 b
S5	0.15 c	10.6 cd	6.2 d	0.5 c	27.5 ab	100.0 b	99.5 b	1541.3 c	31.3 b
FC	0.10 e	12.3 c	6.3 cd	0.3 e	16.3 c	80.0 d	50.6 cd	1041.3 e	21.3 b

pH, EC and water-soluble nutrients were measured using the 1:5 volume method (substrate: demineralized water = 1:5, v/v). Different lower case letters indicate statistically significant differences in means among eight substrates at each substrate parameter in each column (Tukey's HSD, $p \leq 0.05$, $n = 4$). nd.: non-detectable concentration within the measurement range of the devices. *Miscanthus* x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.2 Substrate particle size and shape distribution

For both particle width and particle length, the curves of particle size distribution (Figure 4.2), their percentile values and the fractions (Table 4.4) showed four groups of miscanthus substrates in the ascending order of particle size as C3 < C5 < (C10, C15, S5) < FC. The curves of C3 and C5 were closer to those of peat and coir: C3 had similar distribution of particle length to peat and coir with difference in medium and coarse fractions, whereas C5 showed similar distribution of particle width to coir. The distribution curves of C10, C15 and S5 were close with small difference. While C10 and S5 had similar particle size distribution, C15 showed difference in the 10^{th} and 90^{th} percentile, particularly in particle length (Table 4.4). Hammermilling miscanthus through a 15-mm screen (C15) produced longer particles (higher d90 value) compared to hammermilling through a 10-mm screen (C10). However, the C15 in this study also showed finer particles than C10, expressed as smaller d10 value. The fraction of fine particles (< 0.5 mm) in C15 was 5% higher than that in C10, but non-significant (Table 4.4). The slight difference

in fine particles between C15 and C10 indicated that hammermilling through screen size from 10-15 mm showed a narrow deviation. The particle length curve of FC had steep slope at size 30 mm, because the measurement range of CamSizer P4 is smaller than 30 mm and FC had about 8% of particle length greater than 30 mm (Figure 4.2).

Non-uniformity coefficient indicates the homogeneity of particle size distribution. Peat and coir had similar non-uniformity coefficients in both width and length (Table 4.4). On the other hand, miscanthus substrates showed different extent of non-uniformity in each dimension. Given smaller non-uniformity coefficients, particle width of miscanthus substrates was more homogenous than particle length. Specific surface area showed the same grouping with reducing particle size increasing specific surface area as peat > coir > C3 > C5 > (C10, C15, S5) > FC (Table 4.4).

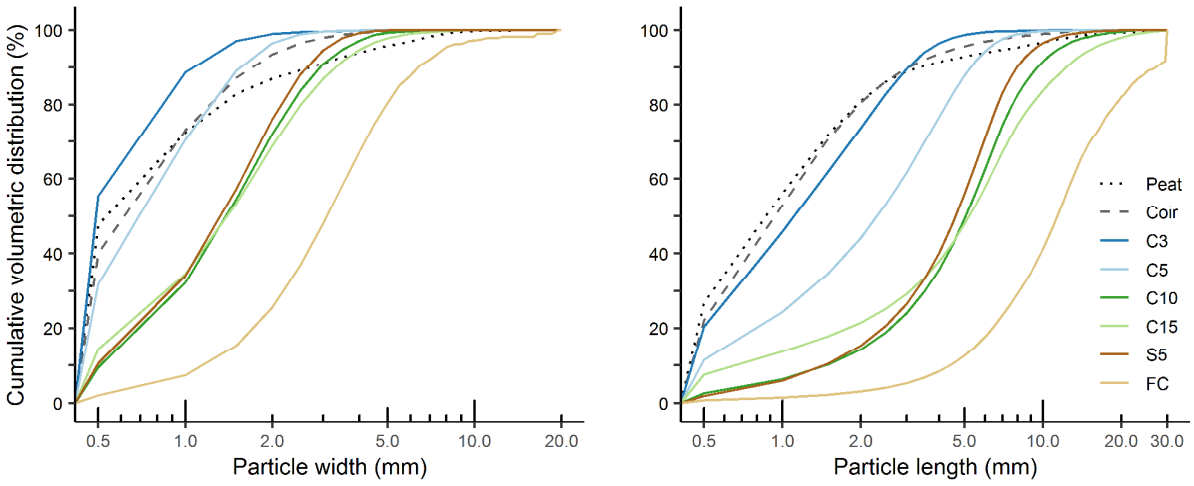


Figure 4.2 Volumetric distribution of particle size of peat, coir and processed miscanthus substrates analyzed with dynamic image analysis. The curve represents for mean value of four replicates of each substrate. *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table 4.4 Volume-based particle size parameters of peat, coir and processed miscanthus substrates using dynamic image analysis

Substrate	Fraction (%) [z]			Percentile values (mm) [y]			Non-uniformity [x]	Specific surface (1 mm^{-1}) [w]
	fine	medium	coarse	d10	d50	d90		
Particle width								
Peat	47.5 ± 6.6 b	39.4 ± 6.3 c	13 ± 0.4 e	0.16 d	0.53 de	2.69 c	4.3 ± 0.3 a	16.3 ± 2.4 a
Coir	40.1 ± 2.0 c	53.4 ± 1.8 b	6.4 ± 0.3 f	0.21 d	0.61 cd	1.68 d	3.6 ± 0.1 bc	12.7 ± 0.6 b
C3	55.4 ± 4.0 a	43.6 ± 4.1 c	1.0 ± 0.2 h	0.17 d	0.46 e	1.04 e	3.1 ± 0.0 cd	15.7 ± 1.1 a
C5	31.9 ± 2.2 d	64.6 ± 1.9 a	3.5 ± 0.5 g	0.23 d	0.70 c	1.53 d	3.6 ± 0.2 b	10.9 ± 0.4 b
C10	9.4 ± 0.6 e	62.7 ± 1.4 a	27.9 ± 1.9 c	0.52 b	1.39 b	2.91 bc	3.2 ± 0.0 bcd	5.2 ± 0.1 c
C15	14.3 ± 1.3 e	54.6 ± 0.4 b	31.1 ± 1.2 b	0.37 c	1.41 b	3.30 b	4.6 ± 0.4 a	6.7 ± 0.4 c
S5	10.5 ± 1.0 e	65.6 ± 0.5 a	23.9 ± 0.8 d	0.49 b	1.33 b	2.61 c	3.2 ± 0.1 bcd	5.1 ± 0.2 c
FC	2.1 ± 0.6 f	23.5 ± 0.7 d	74.4 ± 1.0 a	1.18 a	3.10 a	6.51 a	3.0 ± 0.2 d	2.3 ± 0.2 d
Particle length								
Peat	26.9 ± 6.9 a	54.1 ± 2.8 b	19.0 ± 4.7 f	0.27 d	0.88 e	3.35 d	4.1 ± 0.3 c	22.4 ± 3.9 a
Coir	22.1 ± 2.0 a	58.5 ± 1.0 a	19.4 ± 1.2 f	0.31 d	0.94 e	3.03 d	3.8 ± 0.1 cd	14.6 ± 0.8 c
C3	20.4 ± 3.2 a	53.4 ± 0.7 b	26.2 ± 3.3 e	0.32 d	1.11 e	3.02 d	4.4 ± 0.1 c	18.7 ± 1.3 b
C5	11.5 ± 1.0 b	32.7 ± 0.9 c	55.8 ± 0.8 d	0.45 cd	2.32 d	5.27 d	6.5 ± 0.5 b	13.4 ± 0.5 c
C10	2.6 ± 0.2 c	11.7 ± 0.8 d	85.7 ± 0.7 b	1.47 b	5.06 b	9.50 c	3.9 ± 0.1 cd	6.5 ± 0.2 d
C15	6.6 ± 0.9 bc	13.9 ± 0.7 d	78.5 ± 1.7 c	0.68 c	5.23 b	12.29 b	9.3 ± 1.2 a	9.4 ± 0.6 d
S5	1.9 ± 0.2 c	13.4 ± 0.4 d	84.7 ± 0.6 b	1.45 b	4.63 c	7.95 c	3.6 ± 0.1 cd	6.8 ± 0.1 d
FC	0.8 ± 0.3 c	2.3 ± 0.5 e	96.9 ± 0.9 a	4.39 a	11.38 a	26.92 a	3.0 ± 0.2 d	2.6 ± 0.2 e

[z] Particle size: fine (< 0.5 mm), medium (0.5–2.0 mm) and coarse (> 2.0) (Drzal et al., 1999)
[y] d10, d50 and d90 represent the value of particle size when the portion of particles with size smaller than that value is 10%, 50% and 90%, respectively.
[x] Non-uniformity is d60/d10 (CamSizer's parameter: U3)
[w] Specific surface is the ratio of surface of all particles and volume of all particle (CamSizer's parameter: Sv)
Different lower case letters indicate statistically significant differences in means among eight substrates at each substrate parameter in each column (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 4).
Miscanthus x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Substrates showed different particle shape profiles as in the distribution curve (Figure 4.3) and the mean values at each size category (Table 4.5). Miscanthus substrates had more elongated particles than peat and coir, expressed as the aspect ratio. Among miscanthus substrates, only C3 showed less elongated shape, others had same degree of elongation (Figure 4.3). In all substrates, the degree of elongation increased with the increase in particle size, which was expressed as mean value of aspect ratio at each size category (Table 4.5).

The sphericity parameter (SPHT) describes the sphere shape and also indicates the roughness of particle surface (Miller & Henderson, 2011). The higher SPHT values, the smoother the particle surface. Among the tested substrates, coir had more particles with smoother surface than peat and miscanthus (Figure 4.3, table 4.5). The shredded S5 showed less rough surface than hammermilled miscanthus substrates, particularly in coarse particles. The convexity (Conv), another indicator for surface roughness, also showed the same pattern for miscanthus substrates. Tested substrates had rather symmetrical particles.

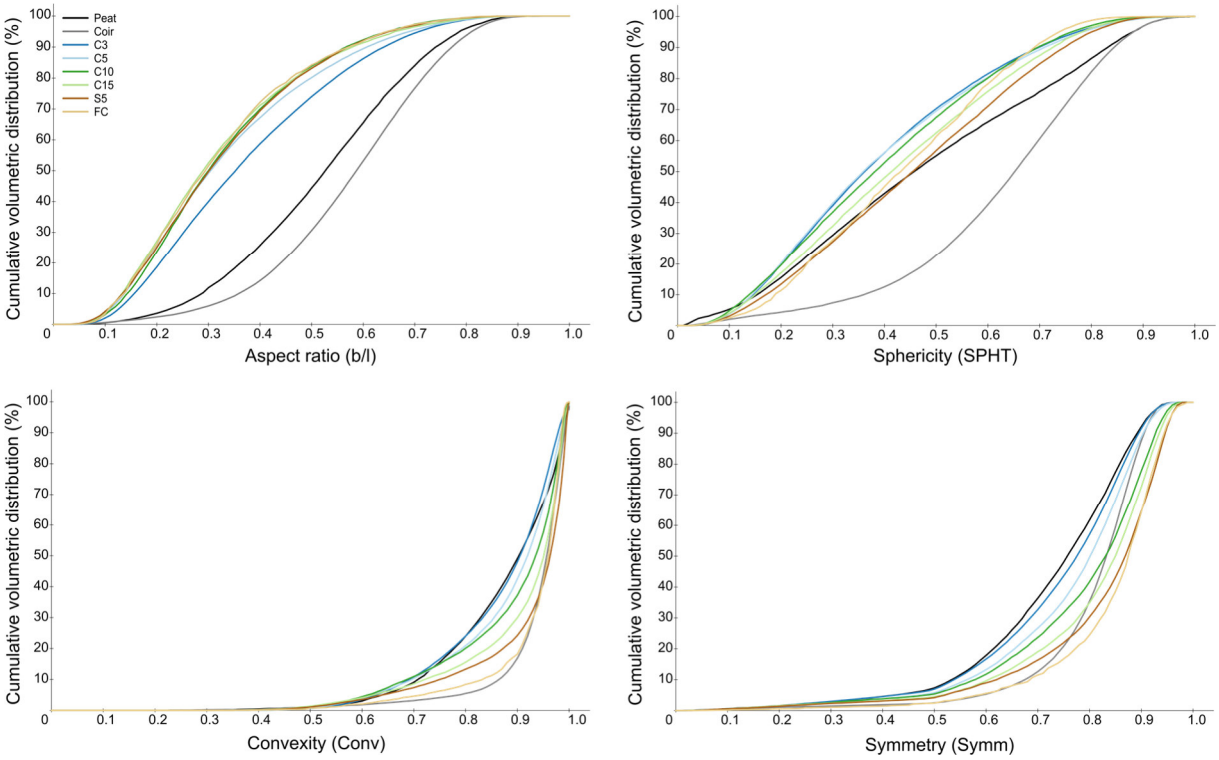


Figure 4.3 Volumetric distribution of particle shape of peat, coir and processed miscanthus substrates analyzed with dynamic image analysis. The curve represents for one measurement of each substrate. *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table 4.5 Volume-based particle shape parameters of peat, coir and processed miscanthus substrates using dynamic image analysis

Substrate	Aspect ratio (b/l)			Sphericity (SPHT)			Symmetry (Symm)		
	fine	medium	coarse	fine	medium	coarse	fine	medium	coarse
Size fractions based on particle width									
Peat	0.51 b	0.57 b	0.58 a	0.47 d	0.50 b	0.46 ab	0.70 e	0.72 c	0.73 bc
Coir	0.62 a	0.60 a	0.59 a	0.69 a	0.63 a	0.48 a	0.82 a	0.82 a	0.81 a
C3	0.49 bc	0.48 c	0.52 b	0.50 cd	0.33 e	0.10 d	0.75 d	0.67 d	0.62 d
C5	0.47 cd	0.40 d	0.49 b	0.53 bc	0.39 d	0.25 c	0.78 c	0.72 c	0.62 d
C10	0.42 e	0.34 e	0.48 bc	0.52 bc	0.41 cd	0.34 bc	0.80 bc	0.77 b	0.70 cd
C15	0.49 bc	0.33 f	0.43 cd	0.58 b	0.42 cd	0.26 c	0.80 ab	0.78 b	0.65 cd
S5	0.37 f	0.35 e	0.52 b	0.47 d	0.46 bc	0.47 a	0.79 bc	0.80 a	0.75 ab
FC	0.45 de	0.32 g	0.41 d	0.53 bc	0.42 cd	0.40 ab	0.80 b	0.81 a	0.77 ab
Size fractions based on particle length									
Peat	0.49 cd	0.35 b	0.31 a	0.59 cd	0.39 d	0.19 de	0.76 d	0.66 e	0.50 e
Coir	0.60 a	0.47 a	0.23 b	0.74 a	0.56 a	0.20 d	0.84 a	0.76 b	0.60 d
C3	0.47 de	0.27 d	0.28 a	0.58 d	0.37 e	0.15 e	0.78 c	0.72 d	0.65 c
C5	0.46 de	0.27 d	0.16 c	0.61 cd	0.40 d	0.22 cd	0.81 b	0.74 c	0.72 b
C10	0.46 de	0.28 d	0.19 c	0.63 bc	0.43 c	0.24 bc	0.83 ab	0.76 ab	0.73 b
C15	0.50 b	0.27 d	0.18 c	0.66 b	0.42 c	0.20 cd	0.83 ab	0.76 b	0.69 c
S5	0.45 e	0.28 d	0.15 c	0.61 cd	0.42 c	0.26 b	0.83 ab	0.76 b	0.77 a
FC	0.50 bc	0.32 c	0.22 b	0.63 bc	0.46 b	0.32 a	0.82 ab	0.76 a	0.79 a

Particle size: fine (< 0.5 mm), medium (0.5–2.0 mm) and coarse (> 2.0) (Drzal et al., 1999)
Different lower case letters indicate statistically significant differences in means among eight substrates at each substrate parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 4). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.3 Substrate hydrological properties: porosity and wettability

3.3.1 Porosity

Porosity of miscanthus substrates showed similar grouping behavior as those in particle size. In the descending order of particle size, miscanthus substrates showed an increased water holding capacity (WHC) and reduced air-filled porosity (AFP). Miscanthus substrates groupings showed descending order of WHC as C3 > C5 > (C10, C15, S5) > FC (Figure 4.4). As finest particle, C3 had similar WHC to commercial peat substrate and slightly lower than commercial coir. Miscanthus substrates had higher AFP compared to peat and coir. Regarding to WHC, miscanthus substrates, except FC, provided WHC in the recommended range of 45-65% for nursery substrates (Yeager et al., 1997). Except C3, other miscanthus substrates had AFP higher than recommended range of 10-30%.

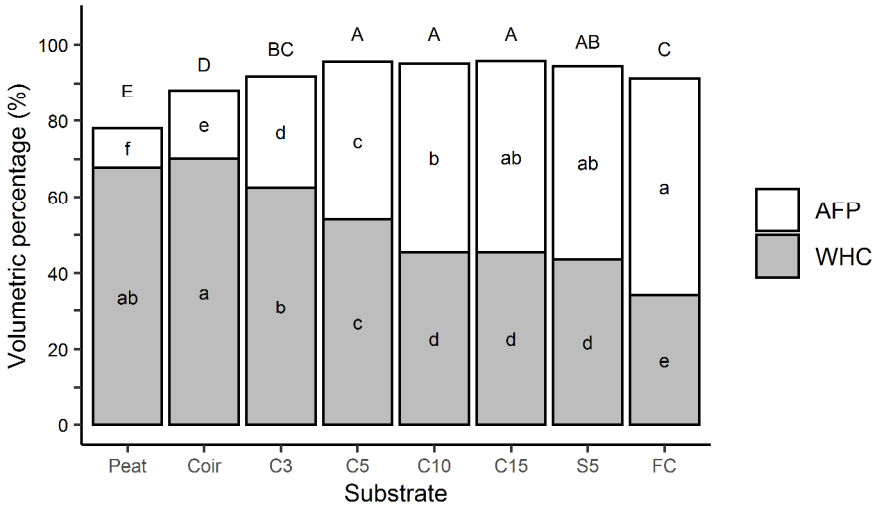


Figure 4.4 Porosity of peat, coir and processed miscanthus substrates: water holding capacity (WHC), air-filled porosity (AFP) and total porosity (as the total height of stacked bars of WHC and AFP).
Different lower case and capital letters indicate statistically significant differences in means among eight substrates at each substrate parameter WHC, AFP and total porosity, respectively (Tukey's HSD, $p \leq 0.05$, n=8).
Miscanthus x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.3.2 Wettability

According to the wettability classes described by Michel et al., (2017), our data showed that the two commercial coir and peat substrates were hydrophilic and miscanthus substrates had low and reversible risk of hydrophobicity (Table 4.6). Miscanthus substrates at higher initial moisture could recover their water contents close to their water holding capacity, but those at lower initial moisture recovered quite slowly but reversibly. As hydrophilic material, coir at different IMC values reached 90% of its maximum water retention after the first hydration event (Figures 4.5 and 4.6). Wettability of peat reduces at low moisture content, but amendment of 30% clay as in this commercial peat substrate improved wettability of peat. Based on hydration speed, initial hydration efficiency and retention efficiency, miscanthus substrates could be grouped according to ascending order of risk of hydrophobicity as C3 < C5 < (C10 and C15) < (S5 and FC) (Table 4.6). In general, finer miscanthus substrates showed lower risk of hydrophobicity, except the shredded S5.

Hydration curves of nonwet miscanthus behaved differently compared to nonwet peat and coir (Figures 4.5 and 4.6). Although the nonwet peat and coir had higher IMC than 25%, the nonwet materials reached their maximum water retention slower than the IMC25. Contrarily, the nonwet miscanthus with lower initial moisture content (10%, w) reached their maximum water retention faster than their IMC25 (even for finer substrates such as C3 and C5, the nonwet material retained water as quick as their IMC67 and ICM50). This could be possibly explained by the higher packing bulk density of nonwet miscanthus (Supplementary, figure S4.2) or potentially a hysteretic effect of miscanthus substrates (Benoit & Ceustermans, 2004).

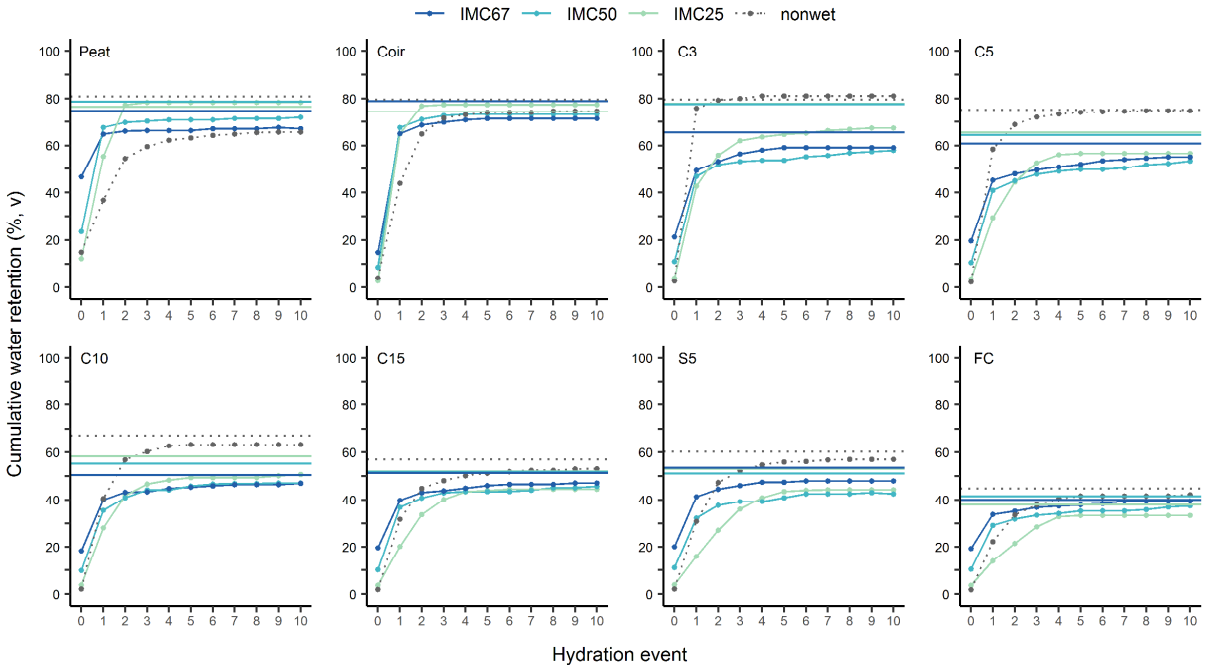


Figure 4.5 Hydration curves of peat, coir and processed miscanthus substrates at different initial moisture content
The curves are cumulative water retention (volume) after 10 successive hydration event. The reference lines are water holding capacity of the substrates. Each data point is a mean of 4 replicates. *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

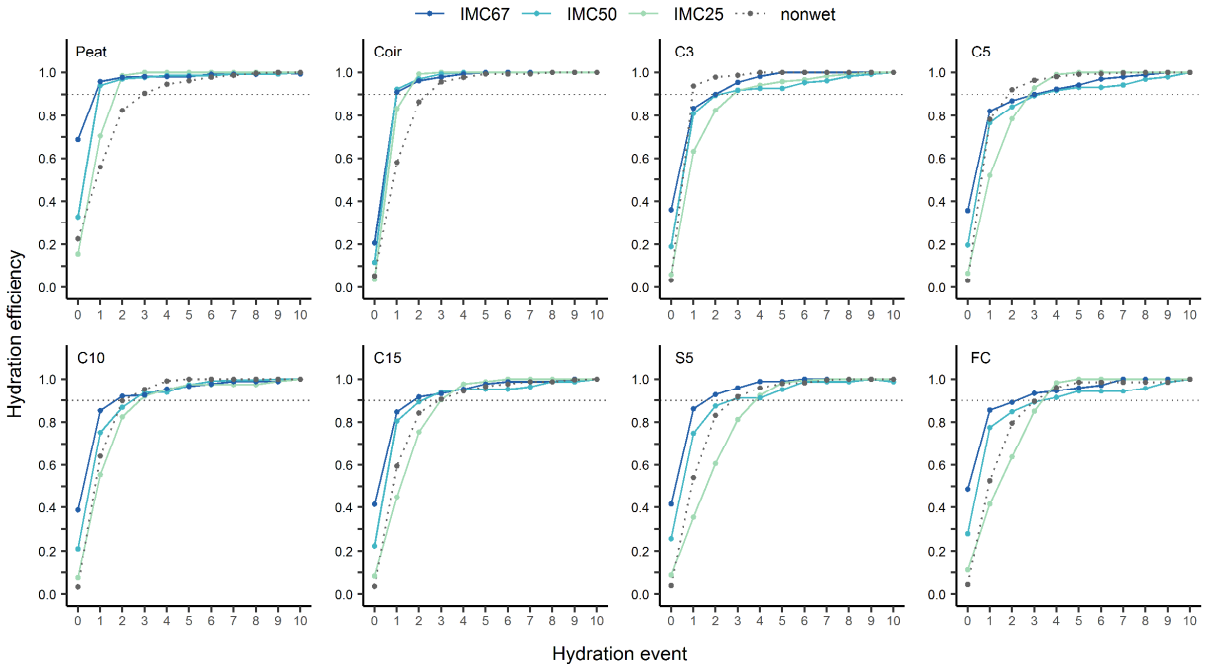


Figure 4.6 Hydration efficiency curves of peat, coir and processed miscanthus substrates at different initial moisture content (IMC)
In each curve, each point represents for the ratio of cumulative water retention at each event to the maximum water retention within 10 events (hydration efficiency). The dotted reference line represents for hydration efficiency of 90%. Each data point is a mean of 4 replicates. *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table 4.6 Hydration efficiency of peat, coir and processed miscanthus substrates

Substrate	IMC [z]	HS [y]	HE1 [x]	RE [w]	Swelling [v]	Risk level of hydrophobicity [u]
Peat	67	1	0.96 a	0.90 b	0 ± 0	no risk of hydrophobicity, but less hydrophilic than coir
	50	1	0.94 a	0.92 b	0 ± 0	
	25	2	0.71 b	1.03 a	2.6 ± 2.1	
	nonwet	3	0.56 b	0.81 c	-1.2 ± 2.4	
Coir	67	1	0.91 a	0.91 b	0 ± 0	no risk of hydrophobicity
	50	1	0.92 a	0.93 b	0 ± 0	
	25	2	0.83 a	1.04 a	6.0 ± 1.1	
	nonwet	3	0.58 b	0.94 b	0.8 ± 1.7	
C3	67	2	0.83 a	0.90 b	0 ± 0	low and reversible risk, least hydrophobic among tested miscanthus substrates
	50	2	0.81 a	0.75 c	0 ± 0	
	25	3	0.63 b	0.87 b	0 ± 0	
	nonwet	1	0.94 a	1.02 a	1.2 ± 1.4	
C5	67	3	0.82 a	0.91 b	0 ± 0	low and reversible risk, 2nd less hydrophobic among tested miscanthus substrates
	50	3	0.77 a	0.83 b	0 ± 0	
	25	3	0.52 b	0.86 b	0 ± 0	
	nonwet	2	0.78 a	1.00 a	2.4 ± 1.7	
C10	67	2	0.85 a	0.93 a	0 ± 0	low and reversible risk, 3rd hydrophobic among tested miscanthus substrates
	50	3	0.75 ab	0.85 b	0 ± 0	
	25	3	0.55 c	0.87 b	0 ± 0	
	nonwet	2	0.64 bc	0.94 a	5.1 ± 3.0	
C15	67	2	0.85 a	0.92 ab	0 ± 0	low and reversible risk, 3rd hydrophobic among tested miscanthus substrates
	50	2	0.81 a	0.88 ab	0 ± 0	
	25	3	0.45 c	0.86 b	0 ± 0	
	nonwet	3	0.59 b	0.93 a	5.5 ± 2.5	
S5	67	2	0.86 a	0.90 ab	0 ± 0	low and reversible risk, 4th hydrophobic among tested miscanthus substrates
	50	3	0.75 a	0.84 b	0 ± 0	
	25	4	0.35 c	0.84 b	0 ± 0	
	nonwet	3	0.54 b	0.95 a	8.0 ± 1.7	
FC	67	2	0.86 a	0.99 ns	0 ± 0	low and reversible risk 4th hydrophobic among tested miscanthus substrates
	50	3	0.78 a	0.91	0 ± 0	
	25	4	0.42 b	0.88	2.6 ± 4.6	
	nonwet	3	0.53 b	0.94	4.0 ± 2.4	

[z] Initial moisture content (w/w)
[y] Hydration speed (HS): number of hydration event applied until substrate reaches 90% its maximum water retention
[x] Hydration efficiency after the first hydration event (HE1)
[w] Retention efficiency (RE): the ratio of maximum water retention to water holding capacity
[v] Swelling (%): proportion of swollen volume after 10 hydration events to the initial volume of substrate column
[u] Risk of level of hydrophobicity according to the category described by Michel et al., 2017
Different lower case letters indicate statistically significant differences in means among four initial moisture content at each substrate in each column (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 4). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.4 pH buffering capacity

Changes in substrate pH to the amount of proton added strongly fitted a linear regression model ($p \leq 0.001$, $R^2 > 0.90$) (Figure 4.7). From those linear regression equations, calculated pH buffering

capacity showed that miscanthus had lower buffering capacity than peat, coir and compost with minimum difference of 0.03, 0.1 and 0.2 mol H^+ kg^{-1}substrate pH^{-1}unit, respectively (Table 7). Tested miscanthus substrates showed similar value of pH buffering capacity, about 0.1 mol proton needed to reduce one pH unit of one kg of substrate. Thus, modifying substrate morphology did not alter pH buffering capacity of miscanthus growing media.

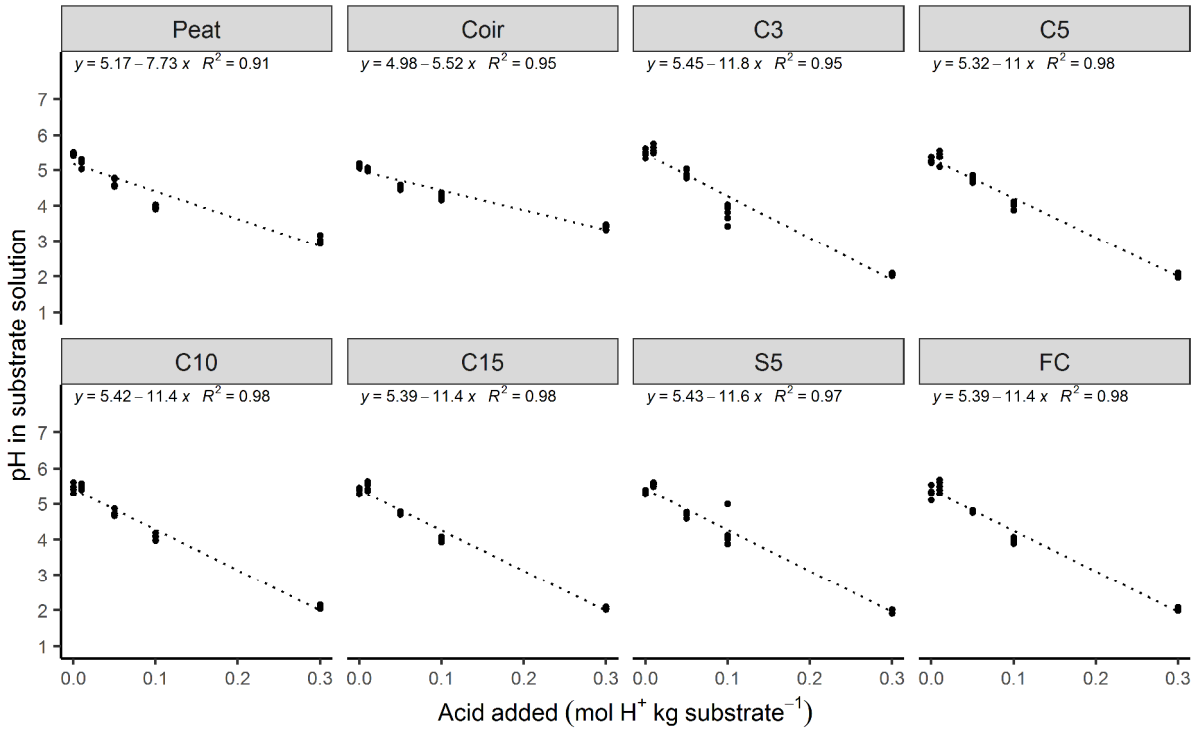


Figure 4.7 Linear regression for the determination of pH buffering capacity of peat, coir and processed miscanthus substrates.
Each point represents for one sample. For each substrate at each proton concentration, there are 5 replicates. *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table 4.7 Initial pH and pH buffering capacity of peat, coir and processed miscanthus substrates

Substrate	Initial pH	pH buffering capacity (mol H^+ kg^{-1}substratepH^{-1}unit) [z]
Peat	6.6	0.13
Coir	6.7	0.18
C3	6.2	0.08
C5	6.3	0.10
C10	6.3	0.09
C15	6.3	0.09
S5	6.2	0.09
FC	6.3	0.09
Compost (different component)	6.4–8.8	0.29–0.45 (Costello & Sullivan, 2014)

[z] pH buffering capacity = -1/slope (slope as fitted slope of linear regression in figure 4.7)
Miscanthus x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.5 N immobilization

All tested substrates showed N immobilization at different values (Table 4.8). NDI was calculated with the N rate of 300 mg N L^{-1}, as N immobilization did not occur in the N rate of 0 mg N L^{-1}. Peat had minimal N immobilization (NDI = 0.9), then coir with NDI of 0.6. All miscanthus had high N immobilization (NDI from 0.2–0.4) with no difference among C3, C5, C10, C15 and S5. The highest N immobilization occurred in coarse FC could be because of its lowest nitrate concentration at day 0. The amount of nitrate concentration in extract solution at day 0 increased with reducing particle size. Moreover, pH in extract solution of miscanthus substrates increased about 0.5 unit after 4 days, while pH in extract solution of peat and coir did not change (Table 4.8).

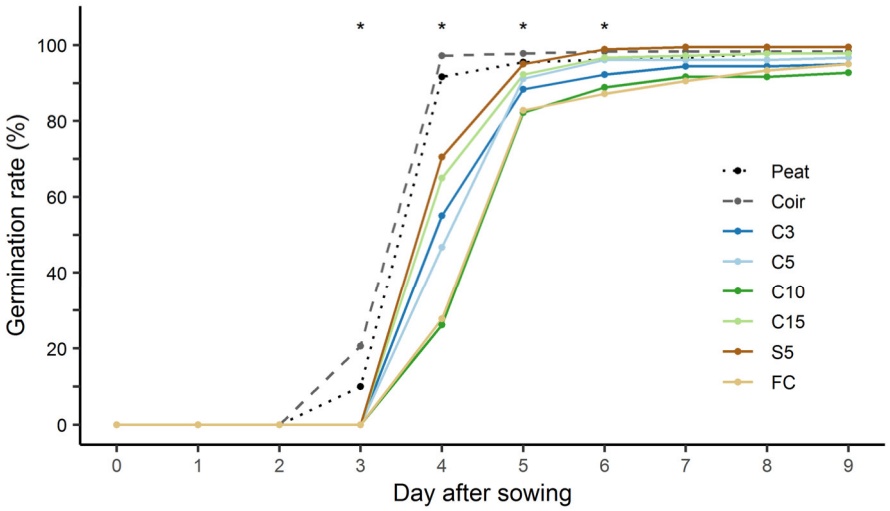
Table 4.8 Nitrogen drawdown index of peat, coir and six processed miscanthus substrates

Substrate	NO_3 concentration in extract solution (mg L^{-1})		pH in extract solution		NDI at day 4 [z]
	day 0	day 4	day 0	day 4	
Peat	294.0 ± 31.6 bcd	260.8 ± 19.7 a	6.5 ± 0.1 b	6.7 ± 0.1 c	0.89 ± 0.12 a
Coir	449.0 ± 60.0 a	261.0 ± 14.9 a	6.6 ± 0.2 b	6.7 ± 0.2 c	0.59 ± 0.11 b
C3	397.5 ± 32.7 ab	174.3 ± 12.8 b	7.1 ± 0.1 a	7.6 ± 0.1 ab	0.44 ± 0.07 b
C5	367.3 ± 110.7 abc	145.8 ± 5.3 bc	7.2 ± 0.1 a	7.7 ± 0 ab	0.43 ± 0.13 bc
C10	296.3 ± 61.6 bcd	113.5 ± 22.9 cd	7.1 ± 0.1 a	7.7 ± 0 ab	0.40 ± 0.13 bc
C15	273.3 ± 22.4 bcd	108.3 ± 12.5 d	7.2 ± 0.1 a	7.7 ± 0 ab	0.40 ± 0.08 bc
S5	249.3 ± 29.9 cd	100.8 ± 15.0 d	7.2 ± 0 a	7.6 ± 0.1 b	0.41 ± 0.11 bc
FC	201.0 ± 45.1 c	34.8 ± 14.1 e	7.2 ± 0 a	7.8 ± 0.1 a	0.18 ± 0.09 c

[z] N drawdown index (NDI) = [NO_3 at day 4]/[NO_3 at day 0], conducted with 300 mg N L^{-1}. Different small letters indicate statistically significant differences in means among substrates at each column (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 4). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

3.6 Growth of Chinese cabbage seedlings

The final germination rate of Chinese cabbage (one week after sowing) was not affected by substrates (Figure 4.8). However, seedling emergence was faster in peat and coir than in miscanthus substrates (day 3, 4, 5 and 6). This could be that the seeds fell deeper in miscanthus substrate due to coarser substrate size, so it took them longer time until the cotyledons showed up on the substrate surface. No significant difference in germination rate was observed among miscanthus substrates. Seeds sown on the coarse FC also germinated as the same rate of other substrates.



Substrate	Peat	Coir	C3	C5	C10	C15	S5	FC
MSET (day)	4.2 bc	4.0 c	4.6 ab	4.8 a	4.9 a	4.6 ab	4.6 ab	5.1 a

Figure 4.8 Germination rate of Chinese cabbage on peat, coir and processed miscanthus substrates. Each point is the mean of germination rate of 4 trays. Each tray consists of 45 seeds. The asterisks at day 3, 4, 5 and 6 represent for significant difference in germination rate among tested substrates (ANOVA, $p \leq 0.05$). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

In extract solution from the sowing cells, pH, EC values and water-soluble nutrient concentration showed different behavior pattern among peat, coir and miscanthus substrates (Figure 4.9). While pH of peat and coir remained around pH of 5.5 during cultivation length of 24 days, pH of miscanthus substrates increased quickly to 8.0 after four days since the start and maintained that value. Among miscanthus substrates, toward the end of cultivation, the finer substrates (C3, C5) tended to have higher pH and EC values than the coarser substrates (C10, C15, S5 and FC) (Supplementary, Table S4.3). Nitrate and calcium concentration in extract solution of miscanthus were much lower than those of peat and coir. Contrarily to low nitrate and calcium concentration, miscanthus substrates had higher concentration of ammonium, potassium and phosphorus compared to peat and coir. No clear difference in those concentration among miscanthus substrates was found. These high concentrations could result from the release from miscanthus feedstock (Table 4.2). Similar pattern was observed in extract solution from sowing cells with seedlings (Supplementary, Table S4.4).

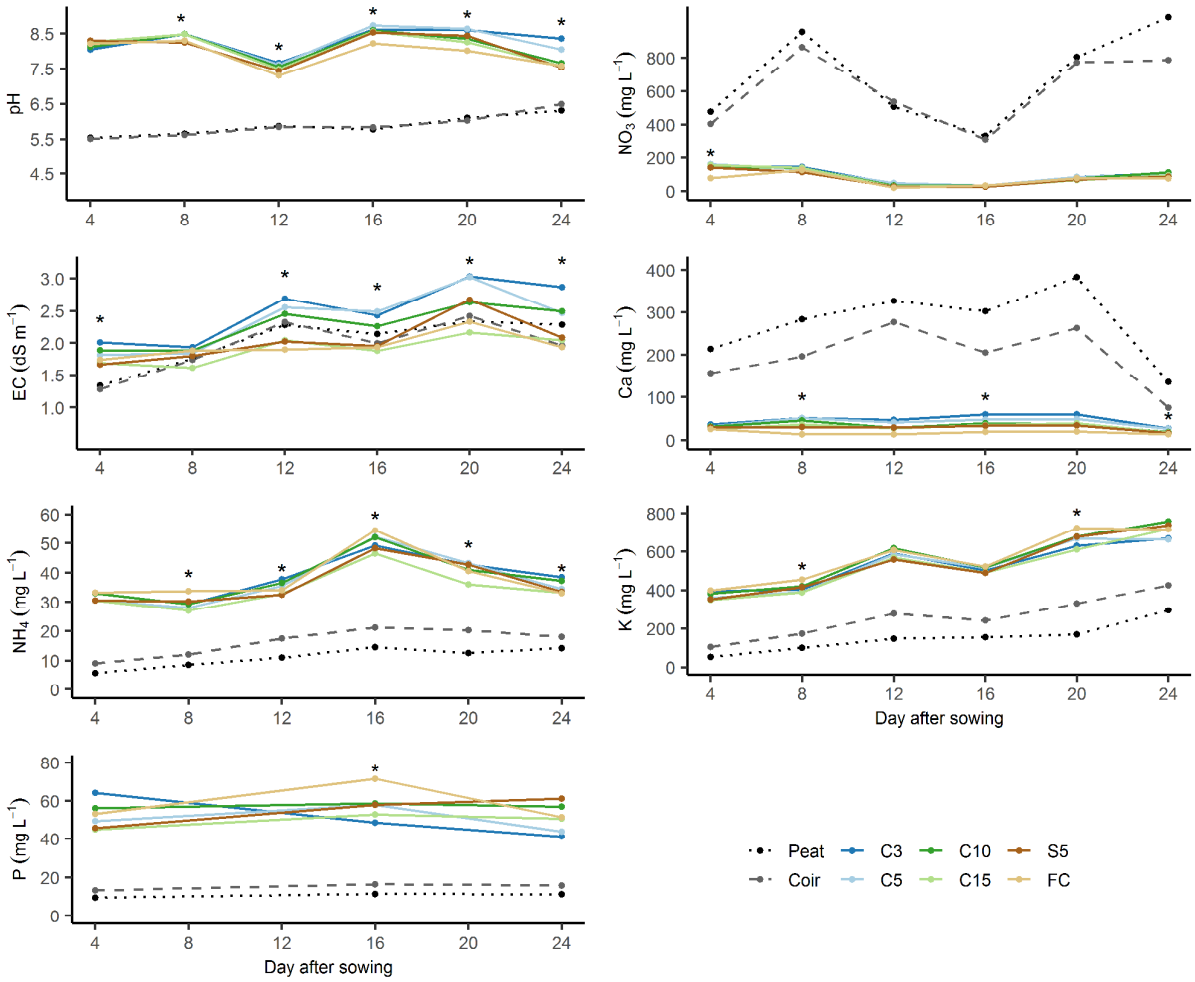


Figure 4.9 pH, EC and water-soluble nutrients concentration in the extract substrate solution (saturation method). For each substrate, one data point represents for the mean of 4 replicates (each replicate consists of 3 sowing cells without having plants). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Seedlings on miscanthus substrates produced less fresh biomass (5 times), dry biomass (2 times) and smaller leaf area (3 times) than those on peat and coir (Figure 4.10). To leaf morphology, seedlings on miscanthus showed smaller and thicker leaves (as leaf mass area) than those on peat and coir. Among miscanthus substrates, seedlings on FC produced the lowest biomass. No difference on seedling growth was found among the rest miscanthus substrates, except the C15 had higher dry seedling biomass. The shapes of violin plots indicated the homogeneous distribution of seedling parameters in each growing media. In general, seedlings on miscanthus substrates developed evenly.

Leaf spectral reflectance showed difference at the wavelength around 550 nm indicating difference in chlorophyll content (Figure 4.11). The ascending order of reflectance was (peat, coir) < C15 < (S5, C10, C5, C3) < FC. As indicator for chlorophyll content, the vegetative indices Carter and Datt were negatively and positively correlated to leaf chlorophyll content, respectively (Main et al., 2011). From leaf spectral reflectance, computed indices Carter and Datt showed that peat and coir had highest chlorophyll content, then C15 and other miscanthus substrates with FC had lowest chlorophyll content.

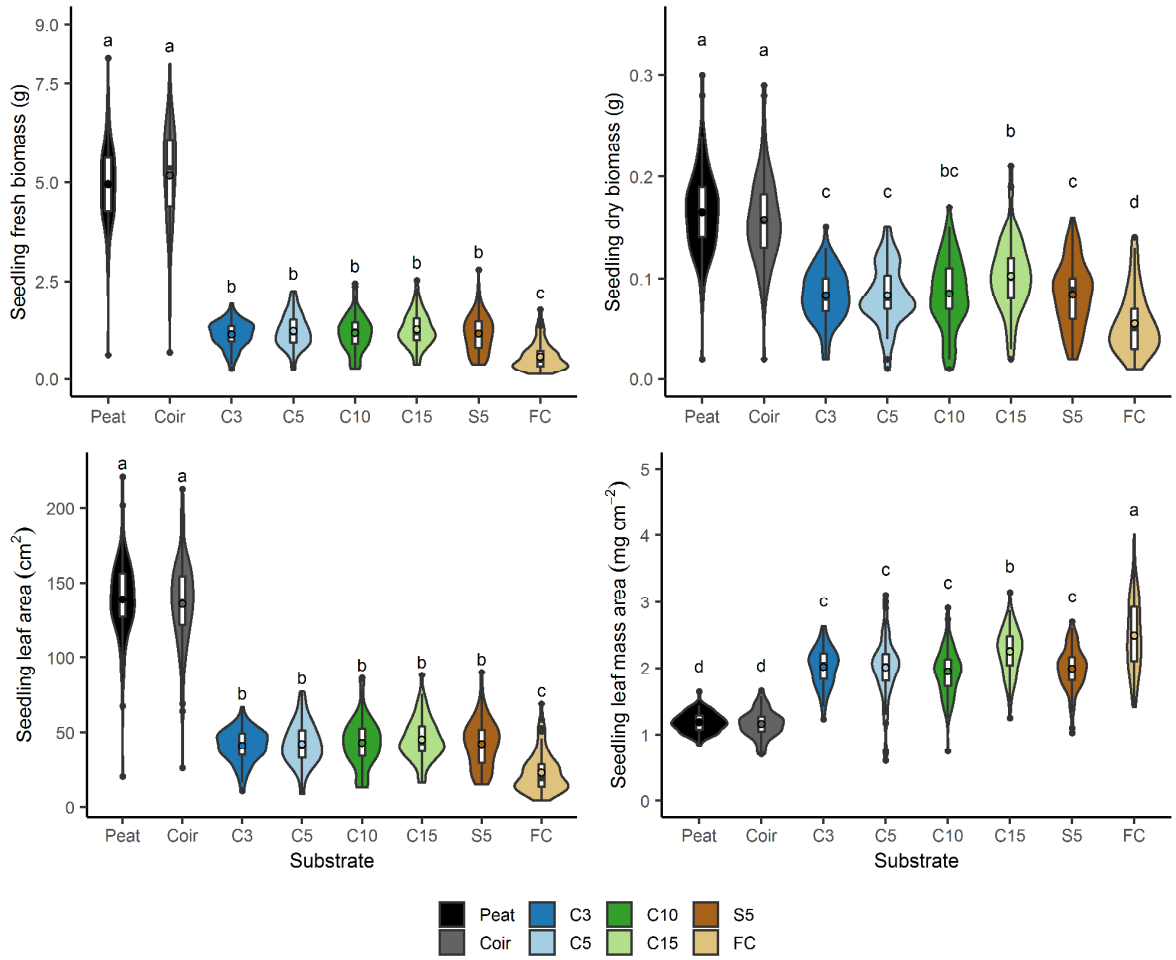


Figure 4.10 Seedling growth on peat, coir and processed miscanthus substrates: seedling fresh biomass, dry biomass, leaf area and leaf mass area.

The violin plot represents the data distribution of 72 single seedlings at each substrate (4 replicates x 18 seedlings). The boxplot inside represents median, interquartile ranges and mean value as point. The width of the violin represents the number of value in each range. Different small letters indicate statistically significant differences in means among substrates (Tukey's HSD, $p \leq 0.05$, mean ± standard, n = 72). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

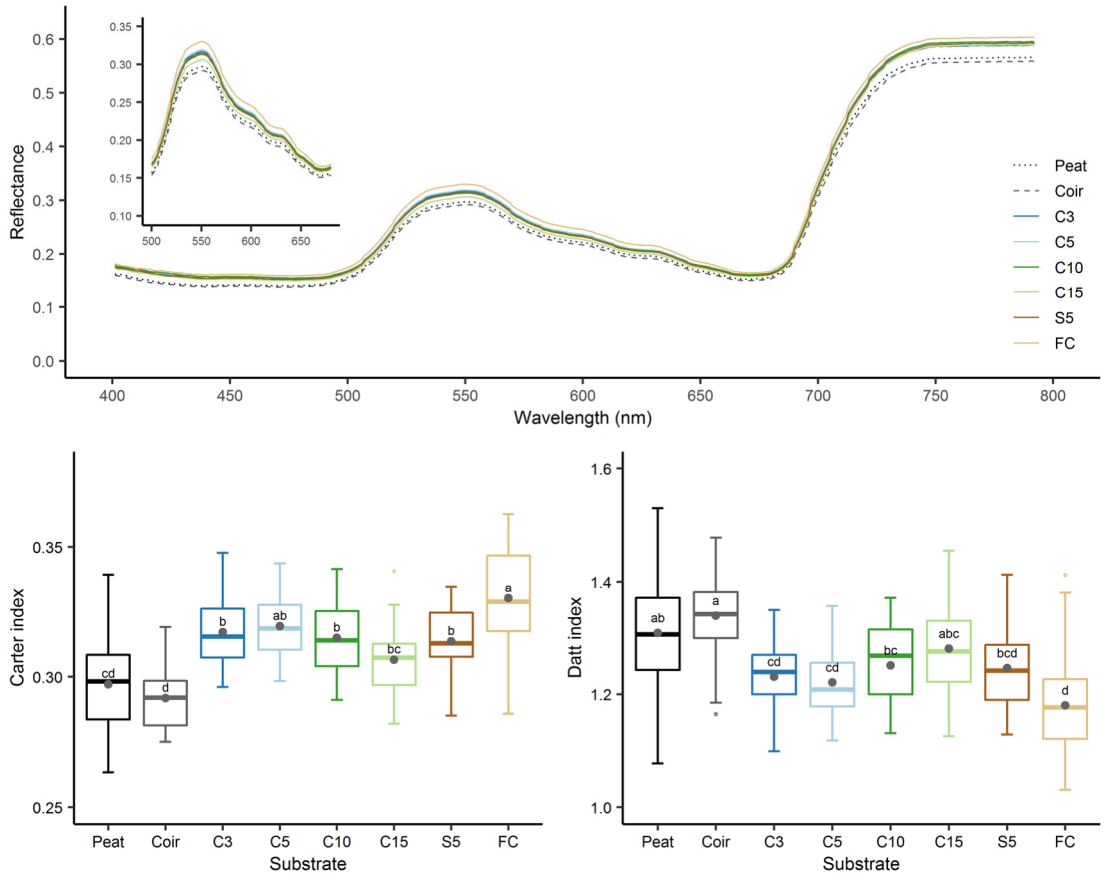


Figure 4.11 Spectral reflectance measured on the first leaf of Chinese cabbage seedlings at day 23 and computed vegetation index Carter6 and Datt4 based on reflectance at 550, 672 and 708 nm.
Different small letters indicate statistically significant differences in means among substrates (Tukey's HSD, $p \leq 0.05$, n = 24). *Miscanthus* x *giganteus* was harvested with a forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

4. Discussion

4.1 Effects of substrate morphology on substrate hydrological properties

Primary mechanical processing of miscanthus created different substrate morphology which strongly influenced porosity properties of substrate. First, regarding particle size, reducing particle size increased water holding capacity in miscanthus and reducing air-filled porosity, following the same grouping pattern in particle size distribution (C3, C5, C10-C15-S5, FC). In the group of C10, C15 and S5, although reducing screen size from 15 mm to 10 mm or processing with different machinery, these substrates showed similar water holding capacity value (~ 45%, v/v). On the other hand, shifting screen size of hammermill from 10 mm to 5 mm and 5 mm to 3 mm showed strong increase in WHC (from WHC of 45% to 54% and 62% in C10, C5 and C3, respectively) (Figure 4.4). In studies on coir, pine tree substrate and bark, increase in WHC mainly resulted from the increase in proportion of fine particle (< 0.5 mm) (Abad et al., 2005; Handreck, 1983; Jackson et al., 2010). Reducing screen size increased the time of particle maintained inside the hammermill, thus increased the proportion of fine particles. We observed that proportion of fine particles (in particle width) was similar among C10, C15 and S5 while it increased three times to five times when shifting screen size from 10 mm to 5 mm and 3 mm,

respectively (Table 4.4). Our data on particle size distribution in volume based using digital image analysis also showed the same tendency to the data in weight based using traditional sieves described by Altland, 2010 and Altland and Locke, 2011 in which miscanthus processed with hammermill equipped with screens from 0.95 cm to 0.48 cm increased its fine particle proportion from 19.4% to 37% and WHC increased 10% in the substrates. Also, the C10, C15 and S5 produced in this study showed their WHC within the lower limit in the acceptance range of 45%-50% with having an amount of around 10% of fine particles. It is in the same finding for pine tree substrate that an amount of 10-15% of fine particles (< 0.5 mm) required to get WHC within the acceptance range (Jackson et al., 2010).

Second, regarding particle shape, the elongated shape of miscanthus increased AFP of substrate. C3 had similar WHC to peat (62% vs 67%), but higher AFP (29% vs 10%). This could be explained by the elongation of substrate particles. C3 had more elongated particles than peat (Figure 4.3, Table 4.5.). This influenced the arrangement of substrate particles resulted in larger pore volume between particles. Moreover, peat substrate was more soft filamentous so it was easier to be bended than C3 leaving smaller space between substrate particles.

Substrate wettability of miscanthus showed the relationship with their particle size and particle shape distribution. In general, miscanthus substrates showed low and reversible risk of hydrophobicity. Hydrophobicity of miscanthus could be explained by the natural waxes which are located mainly on the leaf cuticles and outer stem (Attard et al., 2016). The harvested miscanthus consisted of mainly stem biomass with inner porous hydrophilic parenchyma (Botto et al., 2014; Klímek et al., 2018; Pude et al., 2004) and a small proportion of dried leaf. Reducing particle size could reduce barrier to water absorption into the hydrophilic parenchyma.

In miscanthus, the tendency of particle size showed the tendency of hydrophobicity with reducing particle size reducing hydrophobicity, except the S5 (Table 4.6). Although having same grouping in particle size with C15 and C10 (with slightly smaller size) (Table 4.4), the shredded miscanthus showed higher tendency of hydrophobicity (same group with the coarse FC). It meant that the shredded material could have more barrier to water absorption into the inner parenchyma than the hammermilled substrates. According to particle shape analysis, S5 had smoother surface than hammermilled substrates (Figure 4.3, table 4.5) which could leave less open entrance through the hydrophobic cuticles into the hydrophilic inner part.

In the sowing trays, we observed no hydrophobic effect in miscanthus, except the coarse FC. The moisture in the first week showed no effect on germination rate (Figure 4.8). At the end of growing period, only the FC showed about 1/3-½ top layer dry, and the other miscanthus moistened all the sowing cells (Supplementary, Figure S4.4). C3 and C5 were evenly wet, it could be due to higher capillary rise and less hydrophobic nature of finer particles. At harvest, C10, C15 and S5 showed some particles dried out on the surface but not remarkable.

4.2 Effects of substrate morphology on substrate pH

Substrate morphology showed no effect on pH and pH buffering capacity of fresh miscanthus substrates (Table 4.7). However, in sowing trays, pH in substrate solution of fine substrates (C3, C5) was higher than in coarser substrates (C10, C15, S5 and FC) (Supplementary, Table S4.3). pH in substrate solution of C3 and C5 were significantly higher than that of C10, C15, S5 and FC with the difference of 0.3–0.9 unit at day 24. Altland and Krause, 2009 observed the same pattern for switchgrass substrate with fine substrate (hammermilled with screen size of 0.48 cm) had higher pH than coarse substrate (hammermilled with screen size of 1.25 and 2.5 cm).

According to the pH buffering test, miscanthus had low buffering capacity which was similar to the assumptions of Altland, 2010 and Vandecasteele et al, 2018. However, in the sowing trays, pH in miscanthus substrates shifted quickly from the input pH value of 5.5 to pH 7.5–8 (Figure 4.9). Two assumptions for this pH increase are (1) miscanthus released HCO_3^- from its material, thus increased substrate pH as observed by Guo-jing et al, 2002 in drainage solution of mix miscanthus and wood fiber in tomato cultivation and (2) microbes activity in miscanthus substrates released –OH and caused pH increase as assumed by Domeño et al, 2010 in drainage solution of wood fiber. Regarding to the first assumption on HCO_3^- released from miscanthus feedstock, in a preliminary test on washing substrates, pH of non-washed and washed substrates increased to a similar extent (Supplementary, Figure S4.5). Also, in NDI test, pH in extract solution in miscanthus also increased after 4 days of incubation. Therefore, I assumed that the increase in substrate pH could be from the increase in microorganism activity. Future study should focus on the interaction between microorganism activity (N immobilization) and the evolution of pH substrate.

4.3 Effects of substrate morphology on N immobilization

Substrate morphology showed only difference in N immobilization in coarse material. In general, the coarse FC immobilized about 80% of N applied which was similar to the findings for chopped miscanthus (Vandecasteele et al., 2018). The finer miscanthus C3 and C5 showed similar N immobilization degree to the medium C10, C15 and S5 (about 60% N applied was immobilized) (Table 4.8).

In sowing trays, at very early stage (day 4), nitrate concentration in extract solution in miscanthus reduced to a higher extend to peat and coir. This confirmed a strong N immobilization occurred in miscanthus. Among miscanthus substrates, the finer ones tend to have higher nitrate concentration, but not significantly difference (Supplementary, Table S4.3). This tendency could be due to the higher water holding capacity of finer particles. The ammonium in miscanthus was higher than in peat and coir and remained stable during the cultivation (Figure 4.9). Miscanthus substrates could release a high amount of ammonium from its biomass which contributed to higher ammonium observed in extracted solution (Table 4.3). As there was surplus nitrate, the microbes could prefer to assimilate the nitrate than the ammonium.

4.4 Seedling growth and nutrients available from miscanthus substrates

The seedlings grown on miscanthus produced lower biomass and leaf area than those in peat and coir (Figure 4.10). The seedlings in miscanthus substrates did not show any visual N-deficient symptom such as yellowing leaf, but their leaves were smaller and thicker than those in peat and coir. This could be explained by the lower nitrate concentration and also lower calcium concentration in substrate solution (Figure 4.9), as low calcium concentration could cause stunt growth and restricted leaf development (Bunt, 1988). I assumed that lower calcium concentration in miscanthus substrate solution could be resulted from (1) leaching, (2) binding calcium into the negatively charged sites on miscanthus substrates and (3) precipitation formed between ion Ca^{2+} and PO_4^{3-} in miscanthus substrate solution. According to the preliminary test on cation exchange capacity, miscanthus had low exchange capacity for Ca (Supplementary, Table S4.5). It suggested that just a small amount of calcium might be bounded onto miscanthus surface. The lower calcium concentration in miscanthus substrates could be mainly from leaching effect and precipitation form. Moreover, the high concentration of ammonium, potassium and phosphorus could cause unfavorable condition to seedling growth, especially under low nitrate concentration.

Dry biomass and vegetative indices data showed a tendency that C15 somehow worked better than other miscanthus substrates (Figure 4.11). Because C15 had similar NDI and nitrate concentration in substrate solution to other miscanthus substrates, except FC, I assumed that higher dry biomass of seedlings grown in C15 did not result from less N immobilization, but possibly from a less unfavorable condition caused by less ammonium released from substrate at the very early cultivation (Table 4.3 and S4.3). As the largest substrate after FC, C15 might release slightly less ammonium than other finer substrates (Table 4.3). Although the difference in ammonium might not be noticeable in substrate solution, it might enhance seedling growth in C15. Future study to improve miscanthus performance should focus not only on reducing N immobilization, but also on increasing calcium and reducing ammonium, potassium and phosphorus concentration in miscanthus substrate.

5. Conclusions

Altering substrate morphology via mechanical processing strongly affects substrate hydrological properties and slightly affects substrate pH buffering capacity and N immobilization in miscanthus. Reducing particle size generally increases water holding capacity and reduces air-filled porosity following grouping in proportion of fine particles (< 0.5 mm). Risk of hydrophobicity reduces with reducing particle size in the interaction with roughness of particle surface. Substrate morphology showed no effect on pH and pH buffering capacity of fresh substrate, but the finer substrates tend to have higher pH than coarse substrate in the cultivation. N immobilization was higher in coarse substrate, but similar between medium and fine substrates. While further modifications are required to enhance nutrient availability in substrate solution, this study shows that by selecting proper mechanical processing we could tailor substrate hydrological properties to certain crop's needs.

Supplementary

Figure S4.1 Visual appearance of peat, coir and six processed miscanthus substrates (bar = 1 cm). *Miscanthus* x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table S 4.1 Substrate porosity: wet mass and bulk density of substrates at packing, porosity mean of substrates.

Substrate	Wet mass at packing (g g^{-1})	Bulk density at packing (g cm^{-3})	Total porosity (%)	Water holding capacity (%)	Air-filled porosity (%)
Peat	1.85 ± 0.16	0.263 ± 0.015	78.1 ± 1.8 e	67.7 ± 6.3 ab	10.4 ± 4.9 f
Coir	5.34 ± 0.15	0.086 ± 0.001	88.0 ± 3.6 d	70.2 ± 2.8 a	17.8 ± 6.3 e
C3	3.72 ± 0.06	0.106 ± 0.006	91.8 ± 1.3 bc	62.5 ± 4.1 b	29.2 ± 5.0 d
C5	3.84 ± 0.18	0.099 ± 0.006	95.6 ± 0.8 a	54.0 ± 5.3 c	41.6 ± 5.7 c
C10	3.60 ± 0.22	0.096 ± 0.002	95.2 ± 2.4 a	45.6 ± 2.4 d	49.7 ± 3.9 b
C15	3.65 ± 0.11	0.095 ± 0.002	95.8 ± 1.0 a	45.4 ± 2.1 d	50.4 ± 2.1 ab
S5	3.11 ± 0.11	0.104 ± 0.003	94.5 ± 1.6 ab	43.7 ± 2.0 d	50.8 ± 2.3 ab
FC	2.64 ± 0.10	0.097 ± 0.002	91.3 ± 1.9 c	34.3 ± 2.8 e	57.0 ± 2.7 a

Different lower case letters indicate statistically significant differences in means among tested substrates at each column (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 8). *Miscanthus* x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

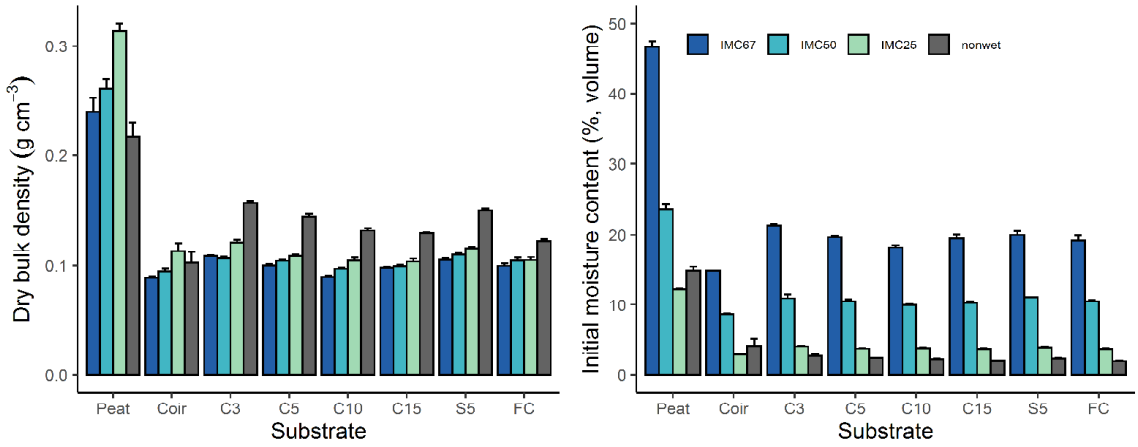


Figure S 4.2 Substrate wettability: packing bulk density, initial moisture content of peat, coir and processed miscanthus substrates.

Miscanthus x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammermill with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

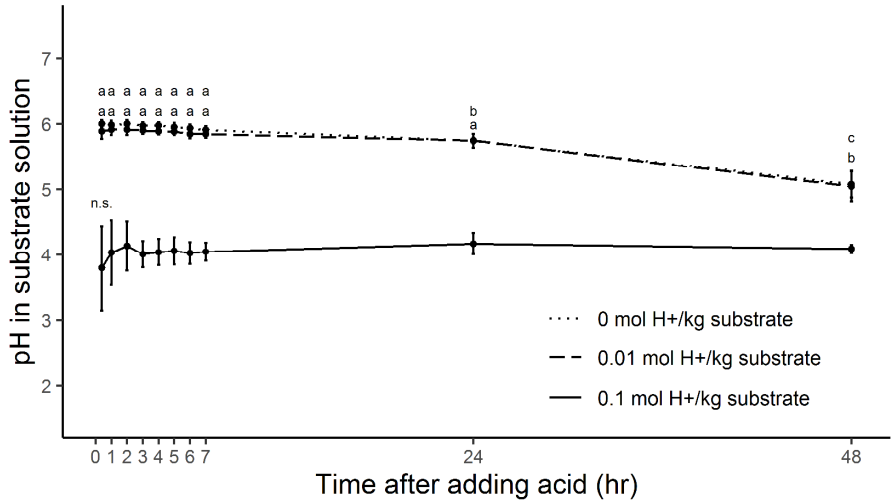


Figure S 4.3 Substrate pH buffering capacity: time to pH stabilization.

The curves represent for mean values of S5 and C3. Different lower case letters indicate statistically significant differences in means among time points at each proton concentration (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 10). The time at 24 hours after acid addition was selected as time when pH stabilization. At 48 hours after acid addition, fungal mycelium on surface of substrate suspension was observed.

Table S 4.2 Nutrient concentration of input nutrient solution for seedling growth

Nutrient		**Concentration**
NH_4	mmol L^{-1}	1.20
K		9.50
Ca		5.40
Mg		2.40
NO_3		16.0
H_2PO_4		1.50
SO_4		4.40
Fe	µmol L^{-1}	15.0
Mn		10.0
Zn		5.0
B		30.0
Cu		0.75
Mo		0.50
EC (dS m^{-1})		2.63
pH		5.5

Table S 4.3 pH, EC and water-soluble nutrients from extract solution of sowing cells without plants

Substrate	day 4	day 8	day 12	day 16	day 20	day 24
pH						
Peat	5.5 ± 0.0 b	5.7 ± 0.0 c	5.9 ± 0.1 e	5.8 ± 0.2 c	6.1 ± 0.1 d	6.3 ± 0.2 d
Coir	5.5 ± 0.1 b	5.6 ± 0.0 c	5.8 ± 0.1 e	5.8 ± 0.1 c	6 ± 0.1 d	6.5 ± 0.2 d
C3	8.0 ± 0.2 a	8.5 ± 0.1 a	7.7 ± 0.1 a	8.6 ± 0.1 a	8.6 ± 0.1 a	8.4 ± 0.1 a
C5	8.1 ± 0.1 a	8.5 ± 0.0 a	7.6 ± 0.1 ab	8.7 ± 0.1 a	8.6 ± 0.1 a	8.0 ± 0.2 ab
C10	8.1 ± 0.1 a	8.5 ± 0.1 a	7.6 ± 0.1 bc	8.6 ± 0.2 a	8.4 ± 0.2 ab	7.7 ± 0.1 bc
C15	8.3 ± 0.1 a	8.5 ± 0.1 a	7.5 ± 0.0 bc	8.6 ± 0.1 a	8.3 ± 0.1 bc	7.6 ± 0.2 c
S5	8.3 ± 0.1 a	8.2 ± 0.1 b	7.4 ± 0.0 cd	8.5 ± 0.1 ab	8.4 ± 0.1 ab	7.5 ± 0.2 c
FC	8.2 ± 0.2 a	8.3 ± 0.1 ab	7.3 ± 0.0 d	8.2 ± 0.1 b	8.0 ± 0.2 c	7.6 ± 0.3 c
EC (dS m^{-1})						
Peat	1.3 ± 0.1 cd	1.8 ± 0.2 a	2.3 ± 0.4 bc	2.1 ± 0.1 ab	2.3 ± 0.1 bc	2.3 ± 0.1 bc
Coir	1.3 ± 0.1 d	1.7 ± 0.1 a	2.3 ± 0.2 bc	2.0 ± 0.1 b	2.4 ± 0.1 bc	2.0 ± 0.0 c
C3	2.0 ± 0.1 a	1.9 ± 0.2 a	2.7 ± 0.1 a	2.4 ± 0.1 a	3.0 ± 0.2 a	2.9 ± 0.3 a
C5	1.8 ± 0.2 ab	1.8 ± 0.1 a	2.6 ± 0.3 ab	2.5 ± 0.3 a	3.0 ± 0.2 a	2.5 ± 0.3 ab
C10	1.9 ± 0.2 ab	1.9 ± 0.2 a	2.5 ± 0.2 ab	2.3 ± 0.1 ab	2.6 ± 0.3 bc	2.5 ± 0.1 ab
C15	1.7 ± 0.1 ab	1.6 ± 0.1 a	2.0 ± 0.2 bc	1.9 ± 0.1 b	2.2 ± 0.2 c	2.0 ± 0.3 bc
S5	1.7 ± 0 bc	1.8 ± 0.1 a	2.0 ± 0.1 bc	2.0 ± 0.3 b	2.7 ± 0.2 ab	2.1 ± 0.2 bc
FC	1.7 ± 0.2 ab	1.9 ± 0.1 a	1.9 ± 0.1 c	1.9 ± 0.1 b	2.3 ± 0.3 bc	1.9 ± 0.1 c
NO_3 (mg L^{-1})						
Peat	478 ± 62.2 a	954.0 ± 138.5 a	506.3 ± 100.1 a	334.3 ± 40.0 a	804.0 ± 48.1 a	1040.8 ± 62.5 a
Coir	405.8 ± 55.3 a	862.0 ± 67.1 a	537.8 ± 116.7 a	312.3 ± 17.5 a	772.3 ± 33.1 a	787.3 ± 60.1 b
C3	149.8 ± 17.9 bc	144.0 ± 25.8 b	44.8 ± 4.3 b	31.3 ± 3.3 b	84.8 ± 10.5 b	106.8 ± 10.8 c
C5	160.8 ± 15.8 b	131.3 ± 17.0 b	48.3 ± 10.8 b	34.8 ± 3.0 b	84.5 ± 6.6 b	103.0 ± 8.7 c
C10	148.0 ± 15.0 bc	138.8 ± 11.9 b	33.5 ± 2.5 b	33.8 ± 2.2 b	76.0 ± 5.7 b	111.8 ± 6.8 c
C15	156.0 ± 7.4 b	140.0 ± 24.8 b	28.5 ± 2.1 b	29.0 ± 1.8 b	66.0 ± 3.5 b	94.5 ± 4.4 c
S5	140.5 ± 23.6 bc	113.3 ± 20.4 b	26.8 ± 1.7 b	26.5 ± 3.7 b	70.0 ± 3.2 b	86.8 ± 8.2 c
FC	78.5 ± 16.8 c	126.0 ± 18.1 b	21.8 ± 1.7 b	34.0 ± 2.2 b	77.0 ± 4.5 b	76.8 ± 3.9 c
NH_4 (mg L^{-1})						
Peat	5.8 ± 0.5 b	8.5 ± 1.9 c	11.0 ± 1.3 d	14.5 ± 1.3 c	12.5 ± 1.0 d	14.3 ± 1.0 c
Coir	9.0 ± 0.8 b	12.0 ± 0.8 c	17.5 ± 1.8 c	21.3 ± 1.0 c	20.3 ± 1.3 c	18.0 ± 0.0 c
C3	33.0 ± 3.2 a	29.3 ± 2.2 ab	37.8 ± 1.3 a	49.3 ± 2.2 ab	42.8 ± 3.7 a	38.5 ± 2.6 a
C5	30.5 ± 2.4 a	28.0 ± 2.2 b	35.5 ± 2.6 ab	52.3 ± 3.6 ab	43.3 ± 2.2 a	34.5 ± 3.7 ab
C10	33.0 ± 2.2 a	29.3 ± 3.9 ab	36.5 ± 2.4 ab	52.0 ± 2.2 ab	41.0 ± 1.8 ab	37.3 ± 1.5 ab
C15	30.5 ± 2.1 a	27.0 ± 2.2 b	32.8 ± 3.3 b	46.5 ± 3.4 b	36.0 ± 2.4 b	33.3 ± 2.5 b
S5	30.5 ± 0.6 a	30.3 ± 2.6 ab	32.5 ± 1.2 b	48.3 ± 5.4 ab	42.8 ± 2.2 a	33.5 ± 1.7 b
FC	33.3 ± 2.5 a	33.8 ± 1.0 a	34.0 ± 2.1 ab	54.5 ± 1.3 a	40.5 ± 3.5 ab	33.0 ± 1.8 b
K (mg L^{-1})						
Peat	53.3 ± 9.0 b	100.8 ± 16.9 d	146.8 ± 22.8 c	154.5 ± 12.7 c	168.8 ± 14.5 d	295.0 ± 16.9 c
Coir	104.0 ± 10.8 b	173.8 ± 14.0 c	277.0 ± 30.6 b	241.5 ± 6.7 b	331.5 ± 25.8 c	424.3 ± 8.2 b
C3	394.8 ± 33.3 a	405.0 ± 27.7 ab	589.5 ± 56.5 a	501.3 ± 10.1 a	629.8 ± 44.9 ab	668.0 ± 29.7 a
C5	361.0 ± 33.2 a	390.3 ± 13.1 b	585.5 ± 36.2 a	515.5 ± 19.7 a	666.5 ± 35.6 ab	661.5 ± 54.6 a
C10	382.0 ± 19.4 a	419.8 ± 51.4 ab	616.5 ± 48.2 a	518.3 ± 20.6 a	677.3 ± 21.4 ab	757.8 ± 33.1 a
C15	349.8 ± 21.2 a	389.3 ± 7.0 b	568.3 ± 52.4 a	490.5 ± 30.3 a	609.5 ± 56.9 b	717.5 ± 37.2 a
S5	352.8 ± 13.8 a	416.0 ± 25.4 ab	559.0 ± 21.0 a	489.3 ± 44.8 a	676.8 ± 6.2 ab	737.0 ± 75.9 a
FC	398.0 ± 35.8 a	455.0 ± 13.4 a	606.5 ± 41.9 a	522.8 ± 9.7 a	723.0 ± 73.4 a	716.0 ± 43.9 a

Ca (mg L^{-1})						
Peat	214.0 ± 31.6 a	214.0 ± 31.6 a	327.5 ± 68.6 a	303.0 ± 22.2 a	383.0 ± 33.7 a	135.0 ± 10.0 a
Coir	156.5 ± 9.6 b	156.5 ± 9.6 b	277.5 ± 48.0 a	205.5 ± 8.2 b	263.0 ± 33.7 b	75.3 ± 2.6 b
C3	36.8 ± 10.3 c	36.8 ± 10.3 c	46.8 ± 20.2 b	59.5 ± 7.5 c	60.0 ± 15.4 c	27.0 ± 2.2 c
C5	32.0 ± 3.3 c	32.0 ± 3.3 c	41.5 ± 8.0 b	47.8 ± 3.0 cd	49.5 ± 4.0 c	26.8 ± 4.0 c
C10	31.5 ± 3.7 c	31.5 ± 3.7 c	28.8 ± 11.1 b	39.0 ± 3.9 de	36.5 ± 9.5 c	18.8 ± 3.4 cd
C15	26.0 ± 3.2 c	26.0 ± 3.2 c	28.3 ± 8.1 b	35.0 ± 8.7 de	39.0 ± 7.9 c	17.8 ± 1.5 cd
S5	29.3 ± 6.2 c	29.3 ± 6.2 c	29.3 ± 4.3 b	33.8 ± 4.3 de	34.3 ± 3.4 c	17.0 ± 4.2 cd
FC	26.0 ± 11.3 c	26 ± 11.3 c	14.3 ± 6.7 b	19.3 ± 3.4 e	20.5 ± 5.8 c	13.8 ± 3.8 d
P (mg L^{-1})						
Peat	9.6 ± 0.8 b	na	na	11.4 ± 0.8 c	na	11.1 ± 0.6 b
Coir	13.2 ± 0.6 b			16.4 ± 0.7 c		15.8 ± 0.5 b
C3	64.2 ± 12.3 a			48.6 ± 4.0 b		41.3 ± 6.6 a
C5	49.4 ± 3.7 a			57.8 ± 1.8 ab		43.9 ± 5.1 a
C10	56.1 ± 7.9 a			58.6 ± 8.8 ab		56.9 ± 7.6 a
C15	44.9 ± 5.3 a			52.8 ± 11.8 ab		50.7 ± 5.1 a
S5	45.8 ± 9.0 a			57.8 ± 12.1 ab		61.1 ± 13.0 a
FC	53.1 ± 7.2 a			71.5 ± 6.0 a		51.4 ± 14.3 a

Different lower case letters indicate statistically significant differences in means among tested substrates at each parameter for each day (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 4). *Miscanthus* x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammer mills with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table S 4.4 pH, EC and water-soluble nutrients from extract solution of cells with plants

Substrate	pH	EC (dS/m)	NO_3 (mg/L)	NH_4 (mg/L)	K (mg/L)	Ca (mg/L)	P (mg/L)
Day 9							
Peat	6.0 ± 0.1 b	1.6 ± 0.2 c	310.8 ± 48.2 a	8.5 ± 1.7 b	97.0 ± 15.7 b	197.5 ± 29.9 a	7.7 ± 2.3 c
Coir	5.9 ± 0.0 b	1.7 ± 0.3 bc	303.8 ± 82.5 a	13.5 ± 1.9 b	205.5 ± 38.4 b	152.5 ± 26.3 b	18.5 ± 4.2 bc
C3	8.1 ± 0.2 a	2.3 ± 0.5 bc	36.3 ± 5.9 b	35.5 ± 5.7 a	613.3 ± 114.8 a	37.8 ± 18.0 c	59.5 ± 22.7 a
C5	8.1 ± 0.2 a	2.5 ± 0.5 a	38.3 ± 11.6 b	38.8 ± 4.4 a	666.0 ± 46.9 a	30.3 ± 9.6 c	66.4 ± 13.0 a
C10	8.0 ± 0.0 a	2.4 ± 0.2 ab	28.5 ± 4.1 b	39.0 ± 1.6 a	683.8 ± 57.3 a	20.8 ± 10.8 c	67.6 ± 22.4 a
C15	8.0 ± 0.1 a	2.2 ± 0.2 bc	24.8 ± 3.6 b	36.0 ± 3.2 a	646.5 ± 35.1 a	17.3 ± 5.6 c	57.0 ± 3.8 a
S5	8.0 ± 0.1 a	1.9 ± 0.1 bc	18.3 ± 2.9 b	32.0 ± 2.4 a	587.8 ± 59.3 a	23.8 ± 11.5 c	50.7 ± 6.9 ab
FC	7.9 ± 0.1 a	2.0 ± 0.2 bc	19.5 ± 2.9 b	35.3 ± 2.1 a	640.3 ± 55.6 a	2.5 ± 7.4 c	59.4 ± 4.8 a
Day 17							
Peat	6.2 ± 0.0 e	2.1 ± 0.2 cd	425.8 ± 94.4 a	10.3 ± 1.0 b	120.3 ± 16.2 c	327.5 ± 34.2 a	9.0 ± 0.4 b
Coir	6.3 ± 0.1 e	1.8 ± 0.1 d	325.0 ± 19.5 b	15.3 ± 1.0 b	216.0 ± 10.4 b	190.0 ± 12.6 b	15.8 ± 1.7 b
C3	8.4 ± 0.1 a	3.0 ± 0.2 a	107.5 ± 11.0 c	37.0 ± 2.4 a	526.5 ± 24.3 a	51.3 ± 10.0 c	57.4 ± 9.5 a
C5	8.2 ± 0.0 ab	2.8 ± 0.1 ab	95.0 ± 7.8 c	36.3 ± 1.0 a	538.5 ± 7.1 a	54.5 ± 9.2 c	63.4 ± 5.2 a
C10	8.0 ± 0.2 bc	2.5 ± 0.6 bc	82.8 ± 13.7 c	35.0 ± 5.8 a	532.0 ± 79.3 a	38.3 ± 13.1 c	76.1 ± 20.5 a
C15	8.0 ± 0.1 c	2.4 ± 0.2 cd	79.5 ± 5.0 c	33.5 ± 1.3 a	521.8 ± 25.6 a	40.3 ± 7.1 c	52.5 ± 4.7 a
S5	7.9 ± 0.0 c	2.6 ± 0.3 bc	80.0 ± 7.0 c	36.0 ± 2.9 a	561.3 ± 45.2 a	40.0 ± 9.3 c	80.7 ± 21.6 a
FC	7.7 ± 0.0 d	2.1 ± 0.1 cd	77.5 ± 10.9 c	33.5 ± 1.3 a	542.8 ± 29.3 a	28.3 ± 8.1 c	67.9 ± 5.6 a

Different lower case letters indicate statistically significant differences in means among tested substrates at each parameter for each day (Tukey's HSD, $p \leq 0.05$, mean ± standard deviation, n = 4). *Miscanthus* x *giganteus* was harvested with forage harvester (FC, field-chips), then processed with a mechanical fraying facility through a 5-mm screen (S5, shreds) or a hammer mills with screen sizes of 15, 10, 5 and 3 mm (C15, C10, C5 and C3, chips).

Table S 4.5 CEC of miscanthus substrates (n = 2)

Substrate	Cation exchange capacity (cmolc kg^{-1})			
	K	Na	Ca	Mg
C15	13.03	0.12	2.21	3.19
S5	12.37	0.16	1.66	2.17

Figure S 4.4 Seedlings at harvest day, from left to right: peat, coir, FC, S5, C15, C10, C5, C3

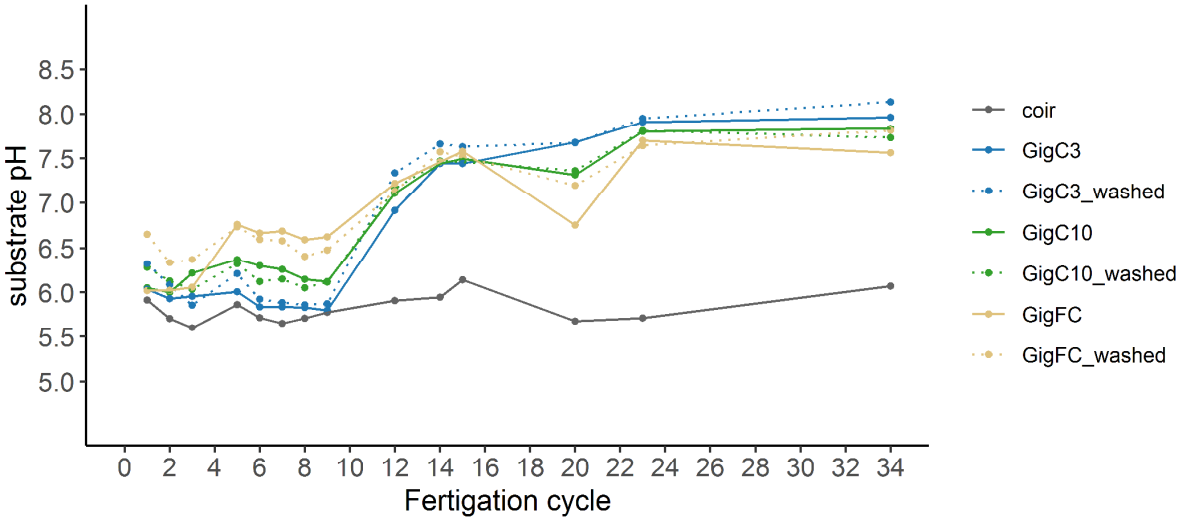


Figure S 4.5 pH evolution of washed and non-washed substrates

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Chapter 5
Effects of washing and steaming pretreatments on reducing nitrogen immobilization in miscanthus substrates

Highlights

- Washing miscanthus substrate with water increased Ca, reduced NH_4, P, K in substrate solution (close to the values in coir) and increased lateral root length of Chinese cabbage seedlings.
- Washing, steaming, primed-steaming and washed-steaming pretreatments did not increase NO_3 in miscanthus substrate solution.
- Steaming delayed germination and reduced lateral root length of the seedlings.
- Washing miscanthus with water showed promising approach.

Abstract

Dry biomass of *Miscanthus* have been studied as soilless substrate for a wide range of horticultural crops showing promising application. N immobilization limits the proportion use of miscanthus in the substrates. This study aimed to quickly evaluate the effect of washing and steaming pretreatments on reducing nitrogen immobilization in stand-alone miscanthus substrate. Both washing treatment (to reduce water-soluble carbon released from miscanthus substrate) and steaming treatments (to reduce initial microorganism population in miscanthus substrates) were hypothesized to enhance plant roots in competing for nitrogen at the very early growth stage of Chinese cabbage seedlings. Results showed that washing, washed-steaming, primed-steaming and steaming did not reduce N immobilization in miscanthus. Steaming delayed seed germination and reduced lateral root length of the seedlings. Washing and washed-steaming treatments did not increase NO_3 concentration in substrate solution, but increased Ca and reduced NH_4, P, K. Seedlings in washed substrate produced longest lateral roots. In conclusion, washing pretreatment showed potential in improving nutrient availability in miscanthus substrate. Washing protocol should be improved to be effective on reducing N immobilization.

Key words: root-zone solution, calcium, nutrients, root morphology, seedlings, Chinese cabbage

Abbreviation: EC (electrical conductivity)

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1. Introduction

Based on the expected increase in world population and living standards, global demand for soilless substrate in 2050 is projected to exceed its recent volume by four times, from 67 Mm^3 in 2017 to 283 Mm^3 in 2050 (Blok et al., 2021). In spite of large area of peatland globally, the supply capacity of peat as horticultural substrate has been restricted by the authorities due to the negative environmental impacts from peat extraction. Taking into account the potential supply capacity of key substrates such as peat, coir and wood fiber etc., to meet the demand, growing media industry will need approximately 65 Mm^3 of new alternative substrate annually globally (Blok et al., 2021). Together with promoting future propagation techniques which would employ less volume of substrate or no substrate at all, searching for new alternative raw materials is a key measure of substrate producers (Röse, 2019).

Dry biomass of miscanthus (*Miscanthus* spp.) is a promising renewable feedstock for soilless substrate due to its potential large supply capacity (Heaton et al., 2010) and ecosystem services (Emmerling & Pude, 2017). Studies on miscanthus substrate showed a wide range of application, from rooting substrate (Bąbelewski & Pancerz, 2018) to substrate for nursery plants (Altland & Locke, 2011; Cárthaigh et al., 1997; Frangi et al., 2012), soft fruits as strawberries (Debode et al., 2018; Kraska & Pude, 2019) and fruit vegetables as tomatoes and cucumbers (Guo-jing et al., 2002; Kraska et al., 2018; Nguyen et al., 2021). In general, miscanthus substrate could partially replace peat or pine bark in container substrates. However, due to high nitrogen (N) immobilization, increasing proportion of miscanthus in the substrates often reduces plant growth (Cárthaigh et al., 1997; Frangi et al., 2012).

N immobilization in miscanthus is caused by high carbon (C) content of the material (Vandecasteele et al., 2018). Moreover, besides the possible contamination with saprophytes during harvest, transportation and storage of miscanthus biomass, abundant endophytic bacterial and fungi have been isolated from miscanthus straw (Beekwilder et al., 2019; Schmidt et al., 2018), which pose a high risk of N immobilization in miscanthus feedstock. Indeed, N immobilization in miscanthus substrate occurs at the start of crop cultivation (Nguyen, 2021; Vandecasteele et al., 2017). As stand-alone propagation substrate for Chinese cabbage seedlings, root-zone solution in miscanthus showed lower Ca, NO_3 and higher NH_4, P, K compared to that in peat and coir (Nguyen, 2021). This imbalance might create less favor condition for root growth, which could exacerbate N immobilization in miscanthus substrate.

In the competition for N between plants and microorganism, the key determinant of the victory is the spatio-temporal distance to the N source (Hodge et al., 2000). Although microbes are often seen as the victor because of their fast metabolism rate, plants can also win when N source is placed near to the plant roots. Altland and Locke (2011) observed less N immobilization in miscanthus substrates when controlled release fertilizer was placed beneath the transplants. Besides adjusting cultivation practices, modifying substrates to create less favorable condition for microorganism could also support plant roots.

This study seeks simple modifications to reduce easily accessible C and the microorganism population from the substrates. While completely removal of C content and microorganism from the

substrates is impractical, reducing the presence of these two factors at the start of cultivation could provide early support to plant roots.

Miscanthus substrates released a considerable amount of water-soluble C (383–913 mg L^{-1}substrate in the 1:5 volume extract), which was easily accessible to microorganism (Vandecasteele et al., 2018). Therefore, washing miscanthus with water might possibly remove a remarkable amount of water-soluble C. Moreover, NH_4, K and P from miscanthus substrates could be also washed out, which might increase Ca in the root-zone solution.

Steaming is a common practice to eliminate plant pathogens, pests and weed seeds in soil and plant biowaste (Bunt, 1988; OEPP/EPPO, 2008). Heat treatment employs the temperature of above 70°C for 1-4 h, preferably by wet heat (OEPP/EPPO, 2008). Treatment effectiveness depends on temperature and duration of the treatment, particle size and moisture content of the materials, and heat tolerance of target organisms. Steaming spent peat or coir-based substrates (100°C for 1-2 min with a subsequent storage at 70°C for 1 h) showed limited effect on microbial biomass and diversity (Vandecasteele et al., 2020). While bacterial biomass and microbiome were reduced in two batches, fungal biomass and composition remained unaffected in one out of two batches.

This study aimed to assess the effectiveness of washing and steaming pretreatments on reducing N immobilization in miscanthus substrates. Washing and steaming pretreatments were hypothesized to increase nitrate concentration in miscanthus substrate for Chinese cabbage seedlings.

2. Materials and methods

2.1 Substrate pretreatments

Miscanthus substrate was obtained from miscanthus stand (*Miscanthus* x *giganteus*) cultivated at Campus Klein-Altendorf, University of Bonn, Rheinbach, Germany. In April 2020, miscanthus culms were harvested at ground level with a forage harvester (Champion C1200, Kemper, Germany). The dry biomass was stored in a protected barn and further processed in April 2021 with a hammermill (Type BHS100, Buschhoff, Germany) through a screen size of 15 mm. Before being used as growing substrate, the hammermilled substrate was then pretreated as described below. Five miscanthus treatments and a commercial coir substrate (Legro, Netherlands) were investigated (Table 5.1).

Table 5.1 List of tested substrates

Substrate	Source	Pretreatment
Coir	Unfertilized coir (Legro, Netherlands)	non-treated
M	*Miscanthus* x *giganteus* hammermilled with screen of 15 mm	non-treated
Mw		washed
Mws		washed then steamed
Mps		primed then steamed
Ms		non-treated then steamed

Washing: Miscanthus substrate was placed in bags with holes and soaked in water in a container (substrate: water of 1 kg: 20 L). Water was changed every one hour. Electrical conductivity (EC), NO_3

and NH_4 concentration were monitored directly in the water right before the water change (Table 5.2). Due to the lack of facility to monitor carbon concentration in washing solution, the decision on stopping washing was based on the change in EC, NO_3 and NH_4 in washing solution. Substrates were washed 4 times. The washed substrate was then air-dried in the greenhouse.

Table 5.2 Electrical conductivity (EC), nitrate and ammonium concentration in washing solution

In wash solution	1st washing	2nd washing	3rd washing	4th washing
EC (dS m^{-1})	0.77	0.19	0.06	0.05
NH_4 (g L^{-1})	0.018	0.010	0.007	0.006
NO_3 (g L^{-1})	0.047	0.016	0.008	0.008

Steaming: Before being steamed, miscanthus substrates were either non-treated, washed or primed. As fungal spores might not be effectively killed by steaming, priming step was added to facilitate spore germination before steaming. Miscanthus substrates were moistened with KNO_3 solution (50 mg N L^{-1}) (substrate: nutrient solution of 12.5 kg: 1 L). Moist substrate was placed into trays to 5-cm layer, covered with holed plastic film to avoid evaporation and ensure aeration. The trays were placed in the greenhouse for 4 days. On day 4, the primed substrate was steamed at their wet status.

For non-treated and washed substrate, substrates were moistened with tap water (60%, v/v) before steaming to ensure heat transfer among substrate particles. Substrates were steamed in a steam wheelbarrow with a volume capacity of 70 L (BEGA Dämpfschubkarre, Friedrich GmbH, Germany). The timer was set for 2 hours. Temperature change inside substrate was monitored via two temperature sensors placed in the middle of substrate and near the substrate surface (Figure 5.1). After being steamed, substrates were air-dried in the greenhouse.

pH, EC and water-soluble nutrients of substrate treatments were measured in a water-extract solution (substrate: water = 1:5, v/v) (DIN EN 13037:2012-01, 2011; DIN EN 13038:2012-01, 2011; DIN EN 13652:2002-01, 2002). pH and EC were measured using a pH meter (pH 3000, STEP Systems GmbH, Germany) and EC meter (FSEC20, MMM Tech Support GmbH & Co. KG, Germany). NO_3, NH_4, K ions were measured with ion-selected electrode (MULTI ISE, Stelzner, Germany). Orthophosphate (P) was measured with the portable VIS spectrophotometer (DR 1900, Hach Lange GmbH, Germany). Calcium (Ca) was measured with Ca meter (LAQUAtwin, Horiba Scientific, Japan). Changes in morphological surface of miscanthus particles were observed under the scanning electronic microscope (Philips XL30 ESEM).

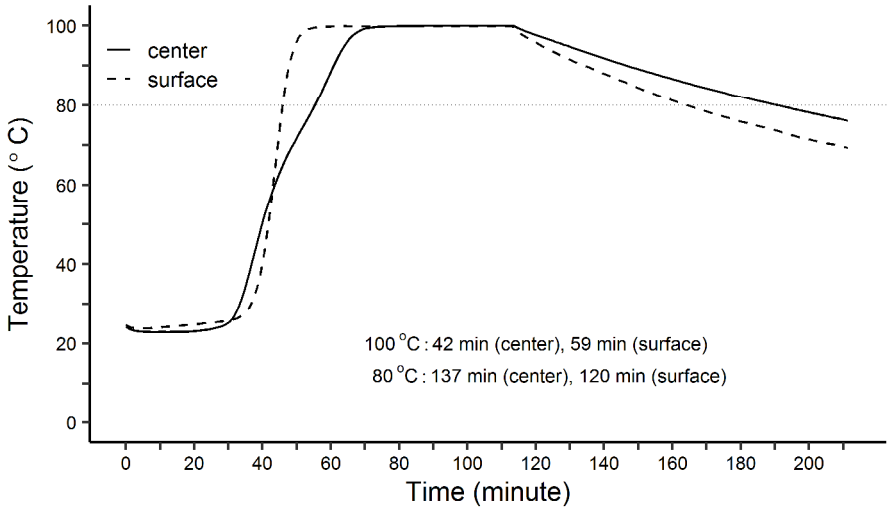

Figure 5.1 Temperature change inside the substrate during the steaming process

2.2 Effects of substrate pretreatments on seedling growth of Chinese cabbage at the early stage

Chinese cabbage (*Brassica rapa* spp. Pekinensi,'Pacifiko F1', Bejo Samen GmbH) was used to test the performance of treated miscanthus substrates under short-term cultivation. Sowing and fertigation were applied as described in Nguyen (2021). Germination rate was recorded daily. To monitor plant growth at early stage of development, seedlings at 11 days after sowing were sampled for fresh biomass and root morphology. Total tap root length and lateral root length were measured using the image processing program ImageJ. Substrates from the sowing cells with and without seedlings were sampled, then extracted with water (saturation method) and measured pH, EC, NO_3, NH_4, P, K and Ca in the extract.

Statistical analysis: The mean difference of tested parameters among substrates was conducted using one-way ANOVA ($p \leq 0.05$), followed by Tukey's HSD multiple comparisons post hoc ($p \leq 0.05$). Statistical analysis was carried out by R software version 4.0.2.

3. Results & discussions

3.1 Substrate characteristics

Washing and steaming pretreatments changed pH, EC and water-soluble nutrients of miscanthus substrates (Table 5.3). Initial substrate pH increased 0.5, 0.2 and 1.8 unit in washed, steamed with pre-washing or non-washing, and primed-steamed substrates, respectively. The rise in pH could be explained by proton washout from miscanthus surface during washing and steaming, which was reported for compost with washing as pretreatment (Bustamante et al., 2021). Washing increased pH higher than steaming, except the primed-steaming. Highest pH increase in primed-steamed substrate could be caused by microorganism growth stimulated by KNO_3 priming and then destruction of microorganism biomass via steaming, which released hydroxyl ions. The rise in substrate pH from 5.0 to 7.0-7.9 had been reported for wood fiber incubated with urea (Lemaire et al., 1989), in wood fiber irrigated with fertilizer solution in the absence of plants (Domeño et al., 2009), and in drained solution of peppers grown in

wood fiber (Benoit & Ceustermans, 1994). Barraud, 1990 as cited in Benoit & Ceustermans, 1994 and Domeño et al., 2009 stated that in wood fiber substrates microorganism consumed nitrates and proportionally emitted hydroxyl ions into the nutrient solution, thus raising the pH. In this study, substrate particles at day 4 of priming showed fungus growth under the microscope.

Washing pretreatment washed out almost all ions accounting for EC. Primed-steaming did not change substrate EC, but steaming alone slightly increase EC of 0.05 dS m^{-1}. Miscanthus substrates released a certain amount of NO_3, NH_4, P, K than coir. Washing pretreatment washed out completely NO_3, 75% NH_4, 80% P and 84% K from miscanthus substrate (Table 5.3). Washed-steamed substrate increased slightly K released compared to washed substrate. Steamed substrates showed surface collapse (Figure 5.2), which would allow chemical release from the materials. This could explain for the increase in NH_4, P, K in steamed substrates. Primed-steamed substrate had similar NO_3 released to steamed miscanthus, which implied that NO_3 from KNO_3 priming was used up by microorganism.

Table 5.3 pH, electrical conductivity (EC) and water-soluble nutrients of miscanthus substrates with steaming and washing as pretreatment and coir

Substrate	pH	EC	Water-soluble nutrient (mg L^{-1}substrate)				
			NO_3	NH_4	P	K	Ca
Coir	5.8 d	0.01 c	3.8 ± 2.5 bc	15.0 ± 0.0 d	8.8 ± 0.8 c	105.0 ± 5.8 e	nd.
M	5.9 d	0.44 b	7.5 ± 2.9 ab	100.0 ± 4.1 b	77.1 ± 8.2 b	2256.3 ± 95.2 b	nd.
Mw	6.5 b	0.01 c	0.0 ± 0.0 c	25.0 ± 0.0 c	13.3 ± 1.1 c	358.8 ± 13.1 d	nd.
Mws	6.1 c	0.05 c	0.0 ± 0.0 c	30.0 ± 0.0 c	13.9 ± 0.5 c	478.8 ± 11.1 c	nd.
Mps	7.7 a	0.41 b	10.0 ± 0.0 a	108.8 ± 2.5 a	95.8 ± 2.2 a	2411.3 ± 33.0 a	nd.
Ms	6.1 c	0.49 a	8.8 ± 2.5 a	102.5 ± 2.9 b	97.9 ± 7.0 a	2382.5 ± 50.7 a	nd.

Different lower case letters indicate statistically significant differences in means among substrates at each substrate parameter in each column (Tukey's HSD, $p \leq 0.05$, n = 4). nd.: non detectable. Coir: commercial unfertilized coir. *M.* x *giganteus* hammermilled through a 15 mm- screen: non-treated (M), washed (Mw), washed-steamed (Mws), primed-steamed (Mps) or steamed (Ms).

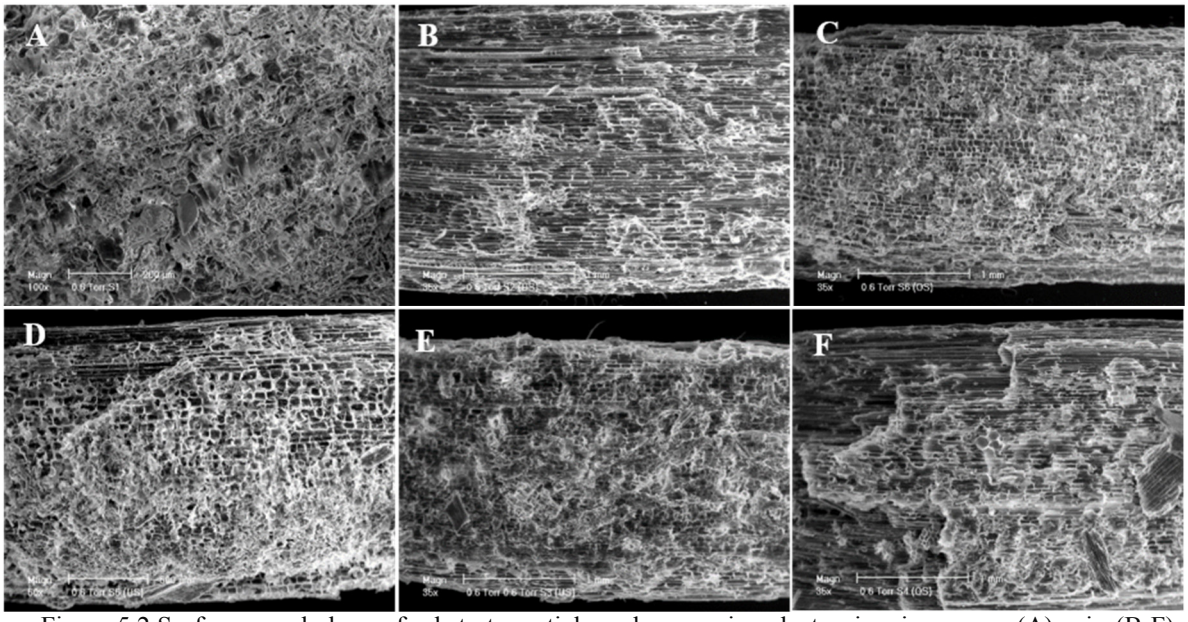

Figure 5.2 Surface morphology of substrate particle under scanning electronic microscope. (A) coir, (B-F) Miscanthus non-treated, washed, washed-steamed, primed-steamed and steamed.

3.2 Effects of substrate treatments on seedling growth of Chinese cabbage at the early stage

Seedlings in miscanthus substrates, except the non-treated and steamed substrates, germinated to the same rate as those in coir (Figure 5.3). Seedlings in steamed substrate particularly had the lowest germination rate.

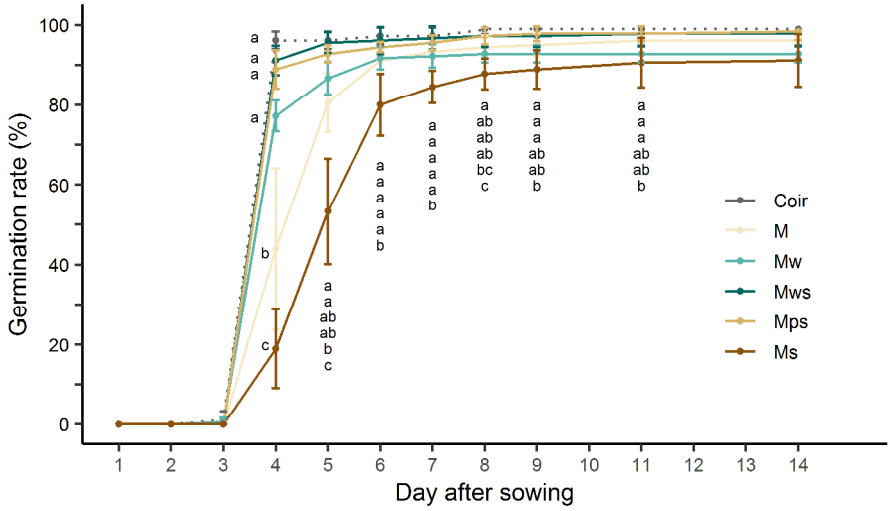

Figure 5.3 Germination rate of Chinese cabbage in the substrates.
Different lower case letters indicate statistically significant differences in means among substrates at each day (Tukey's HSD, $p \leq 0.05$, $n = 4$, each replicate consists of 45 seeds). Coir: commercial unfertilized coir. *M.* x *giganteus* hammermilled through a 15 mm- screen: non-treated (M), washed (Mw), washed-steamed (Mws), primed-steamed (Mps) or steamed (Ms).

At 11 days after sowing, seedlings in coir produced double biomass than those in miscanthus substrates (Figure 5.5). Substrate pretreatments did not have any effect on seedling biomass, except the lowest biomass in steamed miscanthus. Root morphology of seedlings at early stage showed difference among the treatments. The tap root length was similar among the substrates including coir, whereas the length of lateral roots showed difference. Seedlings in washed substrates had similar length of the lateral roots to those in coir and higher than those in non-treated miscanthus and steamed miscanthus. Higher NH_4 and possible phenolic substances released from miscanthus might cause delay germination of seedlings in steamed substrates. In compost, NH_4 and low molecular weight organic acids had been reported to have negative effects on seed germination (Luo et al., 2018).

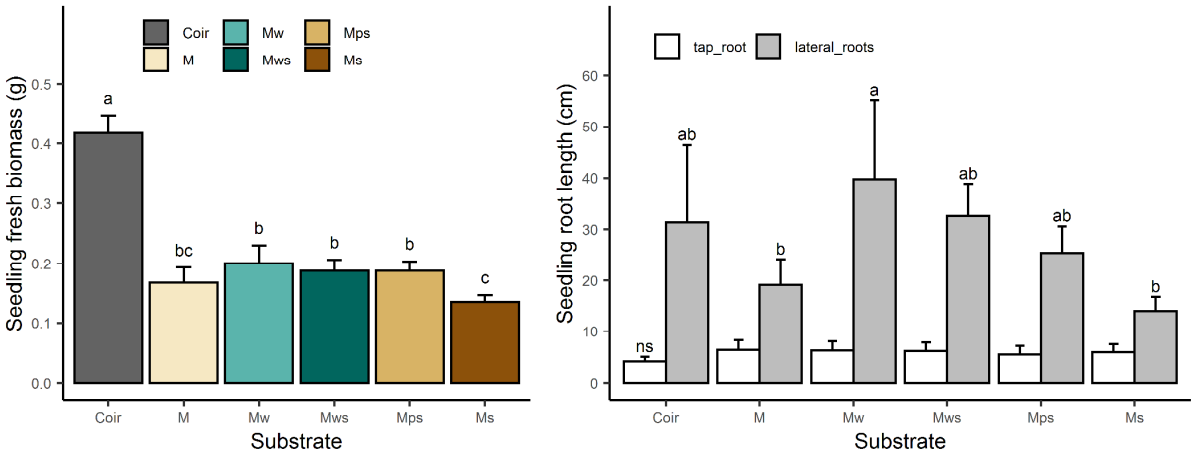

Figure 5.4 Seedling growth at 11-day after sowing (cotyledon stage)
Different lower case letters indicate statistically significant differences in means among substrates at each parameter (Tukey's HSD, $p \leq 0.05$, n = 12 for fresh biomass, n = 5 for root morphology). Coir: commercial unfertilized coir. *M.* x *giganteus* hammermilled through a 15 mm- screen: non-treated (M), washed (Mw), washed-steamed (Mws), primed-steamed (Mps) or steamed (Ms).

In the extraction from both sowing cells with and without seedlings, pH in miscanthus solutions increased to pH 7-8, while that in coir maintained around pH 6 (Figure 5.4). This shift in pH in miscanthus substrates was observed in miscanthus as growbag substrates for tomatoes (Guo-jing et al., 2002; Nguyen et al., 2021) and in miscanthus as propagation substrate for Chinese cabbage seedlings (Nguyen, 2021). Growth of microorganism, particularly saprophytic fungi, on miscanthus substrate was suspected to cause this rise in pH. Washed substrates showed lowest EC, while other treatments had similar EC to that in coir.

Washing and steaming pretreatments did not increase NO_3 in substrate solution compared to non-treated miscanthus and coir (Figure 3.4). All miscanthus substrates had much lower NO_3 than in coir. Washing treatments reduced NH_4 to the similar value in coir. Washed substrates also showed lower K and P than non-washed miscanthus by a factor of 2.7, but the K and P were still nearly double than in coir. However, Ca in washed treatments increased double than non-washed substrates. This increase in Ca could be due to less precipitation of $Ca_3(PO_4)_2$ caused by less P in the solution.

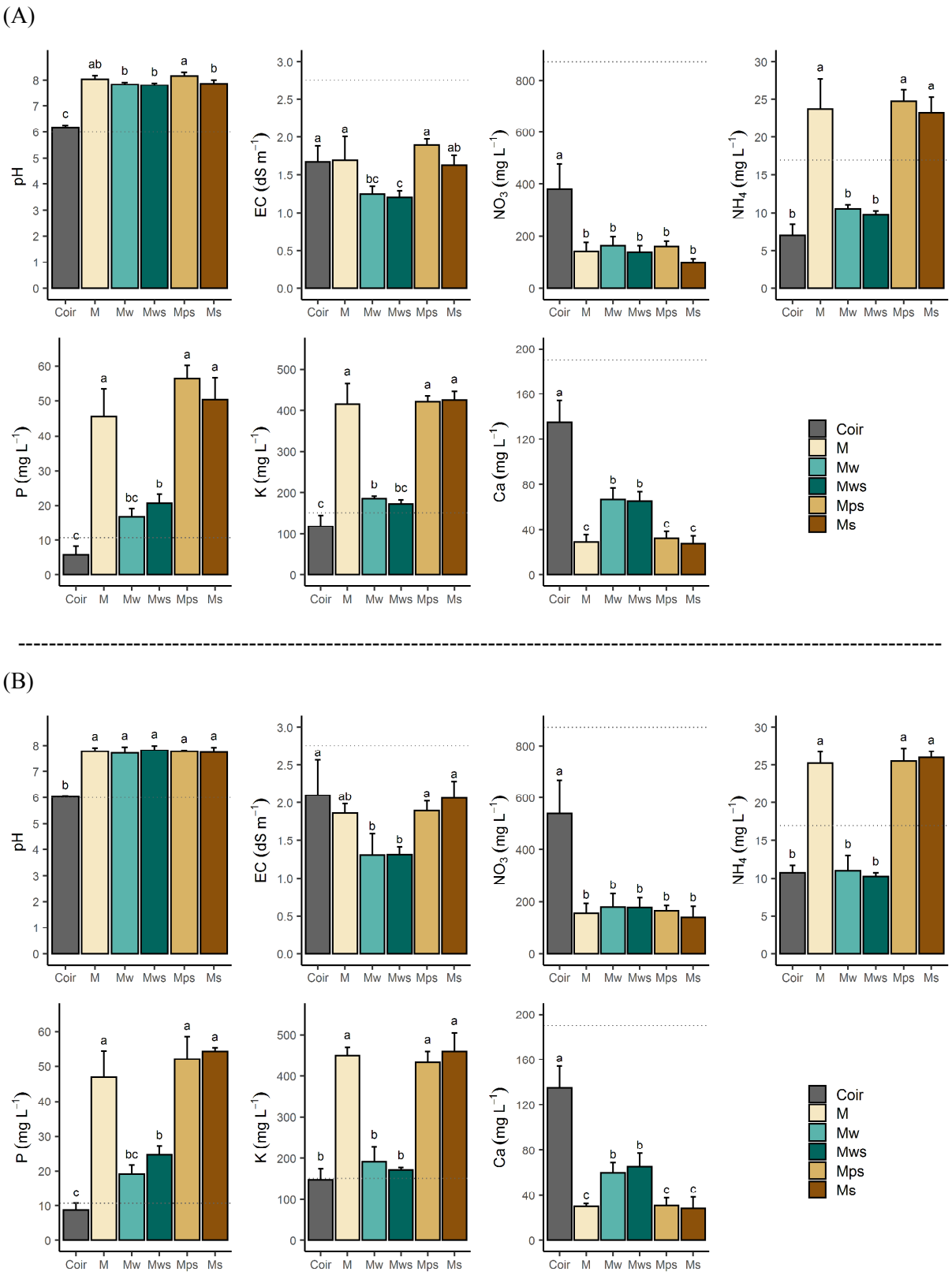

Figure 5.5 pH, electrical conductivity (EC) and water-soluble nutrients in the extracts of substrate solution from the sowing cells with seedling (above) and without seedlings (below) at 11 days after sowing.
The dotted lines represent for values in input solution. Different lower case letters indicate statistically significant differences in means among substrates at each measured parameter (Tukey's HSD, $p \leq 0.05$, $n = 4$).
Coir: commercial unfertilized coir. *M.* x *giganteus* hammermilled through a 15 mm- screen: non-treated (M), washed (Mw), washed-steamed (Mws), primed-steamed (Mps) or steamed (Ms).

4. Conclusions

Washing, steaming and primed steaming could not reduce N immobilization in miscanthus substrates. Although washing pretreatment showed no effect on increasing NO_3 in root-zone solution, it showed potential in increasing Ca and reducing NH_4, K and P in the root-zone solution. Further investigation should focus on monitoring water-soluble C from the washing to improve the washing protocol.

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Conclusion

This PhD work aimed to (i) evaluate potential supply capacity of miscanthus feedstock, opportunities and challenges when developing miscanthus into a commercial substrate by conducting a literature review, and (ii) to optimize substrate performance including increasing water holding capacity, manipulating substrate pH within the optimal range for greenhouse crops and reducing nitrogen immobilization, using the "stand-alone substrate" approach. The results can be summarized as follows:

1. Potential supply capacity of miscanthus feedstock

The availability of the feedstock was estimated based on potential yield of miscanthus (15 t ha^{-1} annually) and potential land availability for bioenergy crops in Europe which is not competing for agricultural activities (1/10 out of 1,300,000 ha estimated land). Theoretically, the potential supply capacity of miscanthus would be 19.5 Mm^3 annually. However, the real supply capacity of miscanthus feedstock depends on the land availability on-site (the proximity to the substrate producers) and the competition for the feedstock from other industries. Given the still relatively small market for miscanthus in Europe, the competition seems not be intense, but uncertain in the future.

2. Opportunities when developing miscanthus into a commercial substrate

Miscanthus feedstock have several promising characteristics for soilless substrates. Due to the plant senescence, the harvest biomass of miscanthus is naturally dried which could benefit the transportation and storage. The efficient nutrient translocation between rhizomes and the above-ground biomass allows quality consistent of the batches between the years. Due to plant resistance to weed, pests and pathogen contamination, miscanthus feedstock has low risk of weed, peat and pathogen contamination. Miscanthus is also potential in enhancing plant strengthening and disease suppressiveness: biogenic Si released from the materials, carrier for biocontrol agent *Trichoderma* spp., abundant beneficial endophytes as pathogen antagonists.

Due to its well-adaptation in the temperate regions, high yield and ecosystems services, miscanthus cultivation is supported by the policies. The ongoing studies on miscanthus agronomy, i.e. plant breeding, plant propagation etc., could benefit the substrate industry by reducing the production cost and increasing the feedstock choice.

3. Challenges when developing miscanthus into a commercial substrate

Miscanthus has high C content with high cellulose and low lignin, which causes N immobilization in the materials. Other limitations in substrate performance are low water holding capacity and high pH, which is often higher than the recommended range for common greenhouse crops. Regarding the feedstock, there has been interests on new applications of miscanthus in other industries which pose the competition in the future for the feedstock.

4. Improving miscanthus performance as growbag substrate for soilless tomatoes

As growbag substrate for tomatoes, miscanthus performed as well as stone wool but lower than coir. Blossom-end rot was the main cause of fruit loss. Miscanthus substrates were hammermilled or shredded without any further pretreatment showing comparable vegetative growth and fruit yield to the standard substrate as stone wool. Although the study did not have tissue analysis and nutrient concentration in root-zone solution except nitrate, comparable vegetative growth of miscanthus suggested that nutrient availability in miscanthus root-zone solution was sufficient to the plants. To improve the performance of miscanthus substrate, further investigation should focus on reducing BER incidence in tomatoes. Learning from the coir substrate, future study could test the coarse material (e.g. the field chips as harvested material from the field) and increasing the growbag height to enhance air supply for plant roots.

After being used as growbag substrate for tomatoes, the used miscanthus substrates could serve as feedstock for direct combustion. The calorific values of used substrates were comparable to the new materials. The increased ash content in used substrates could be reduced by additional washing at the end of cultivation.

5. Improving miscanthus performance via mechanical processing

Water holding capacity of miscanthus substrates could be improved via substrate engineering. Depending on the crops, miscanthus could be hammermilled through a proper screen size (3-15 mm) or shredded using a mechanical fraying facility (5-mm screen). Miscanthus substrate has low risk and reversible risk of hydrophobicity. Hammermilling substrates through a smaller screen size reduced the risk of hydrophobicity.

Miscanthus had initial acidic pH 6-6.3, then increased to 7-8 at the cultivation. pH buffering capacity of miscanthus substrates were low (0.1 mol H^+ per kg substrate to reduce one pH unit) regardless the processing methods. Given the low pH buffering capacity, it seemed to be easy to manage substrate pH. However, when acidifying input nutrient solution in tomatoes (chapter 3), substrate pH in miscanthus growbag still maintained around pH 7-7.5. This suggest that pH rise at cultivation could be from other factors. I assumed that high pH in miscanthus at cultivation could be a consequence from N immobilization. At crop cultivation, microorganism used the nitrates from fertilization solution and assimilated the water-soluble C from miscanthus, then emitted hydroxyl ions, which caused pH rise in the root-zone solution.

Miscanthus had high N immobilization, nearly 60% of applied N was immobilized at the start of cultivation. Processing miscanthus with a hammermill or a mechanical fraying facility did not reduce N immobilization. Besides low nitrate concentration in root-zone solution, miscanthus substrate showed low Ca and high NH_4, K and P, which should be focused in the future investigation to improve nutrient availability in the substrate.

6. Improving miscanthus performance via washing, steaming pretreatments

Although washing and steaming did not reduce N immobilization in miscanthus, washing showed potential in improving nutrient availability in miscanthus substrate. Washing increased Ca and reduced NH_4, P, and K in substrate solution. Chinese cabbage seedlings in washed substrate produced longest lateral root.

Further investigations should focus on the following topics.

- The correlation between the rise in substrate pH and N immobilization. The hypotheses are (1) the activities of saprophytic microorganism in miscanthus would cause the increase in substrate pH; (2) maintaining substrate pH around 6.0 would reduce N immobilization
- Substrate modifications to reduce easily water-soluble carbon from the miscanthus substrate: for example, improving washing protocol, testing defibration techniques in liquid.
- Cultivation management to support plant roots in accessing to N source: for example, N-dose response curve and nutrient recycle.
- Nutrient availability in miscanthus substrates focusing on reducing NH_4, P, K and increasing Ca.
- Plant strengthening (focusing on biogenic Si) and pathogen suppressiveness of the miscanthus substrate (focusing on antimicrobial substances).
- Cascade utilization of the used substrates.

Appendix

Figure A 1. *Miscanthus* x *giganteus* stand before senescence (left) and after senescence (right) at Campus Klein-Altendorf, University of Bonn, Rheinbach, Germany

Figure A 2 Soilless cultivation of tomatoes in growbags in the experiment in 2018 (Chapter 3) at 63 days after transplanting (11.04.2018) (left) and at 134 DAT (21.06.2018) (right): seedlings on stone wool cubes were transplanted onto growbags with 2 plants per bag.

Figure A 3 Fruiting bodies of sac fungi (probably *Peziza repanda*) shown up in miscanthus growbags for soilless tomatoes in the experiment 2018 at 28 days after transplanting (08.03.2018)

Figure A 4 Miscanthus substrate (GigC15) at the end of tomato cultivation (in the experiment 2018): fungus growth shown in the upper layer of the growbag.

Figure A 5 Fruiting bodies of probably *Coprinus cinereus* (left) and the mycelium of probably *Rhizopus* spp. (right) observed in propagation substrate for Chinese cabbage seedlings at 24 days after sowing (Chapter 4)

Figure A 6 Fungal observation under microscope of miscanthus particles at day 4 after incubation with KNO_3 solution (left) and at day 4 after the start of cultivation of Chinese cabbage (middle, right) (Chapter 5)