



and nutrient retention, and thus improved soil quality. With very few exceptions, studies on the effects of biogas on soil properties have consistently noted the improvement of soil quality [23].

Studies on *S. hermaphrodita* have been carried out either under greenhouse conditions [24–26], or under outdoor conditions [7,27]. So far, multi-year field research with *S. hermaphrodita* was mainly carried out in Poland, however, literature on this topic in the English language is limited to a few studies [6,8,28]. To our knowledge, a study focused on the potential of *S. hermaphrodita* for its cultivation on marginal soils and the potential increase of soil fertility via the reapplication of biogas digestates was not performed yet. To close this knowledge gap we designed a combined outdoor mesocosm and field experiment with three different substrates and three different fertilizer regimes, and evaluated plant biomass and soil data over three consecutive years. Additionally, two laboratory experiments were conducted to investigate the effects of digestate fertilization on the water holding capacity and basal soil respiration of a marginal substrate. The underlying research questions and hypothesis were the following:

**Question 1.** What is the yield potential of *S. hermaphrodita* on marginal soils and how does it scale to the yield on conventional field soils? To what extent is yield influenced by fertilization and substrate quality?

**Hypothesis 1.1.** Under optimal conditions, i.e. good soil quality and NPK fertilization, we expect biomass DM yield up to  $20 \text{ t ha}^{-1}$ , based on experiences from earlier field studies [7,8]. Yields from plants grown on substrate of lower quality are expected to be lower, which can partly be compensated by fertilization.

**Hypothesis 1.2.** Plants fertilized with mineral NPK fertilizer will perform better on rich field soil, whereas digestate-fertilized plants will perform better on marginal substrate due to the higher carbon share.

**Question 2.** In how far will digestate fertilization differ in its effect on properties of marginal sandy soil (e.g. soil carbon content, water-holding capacity) from NPK fertilization over numerous growing periods?

**Hypothesis 2.** Digestate fertilization will increase the soil carbon content and by that increase the water holding capacity, soil respiration and the overall yield potential of marginal substrates.

## 2. Materials and methods

### 2.1. Study sites and cultivation

The experimental field site was located at the Research Centre Jülich ( $50^{\circ}53'47''$  north and  $6^{\circ}25'32''$  east; 80 m a.s.l.) and had a size of  $1000 \text{ m}^2$ . In May 2013 a stand of *S. hermaphrodita* (L.) Rusby was established by transplanting pre-cultivated seedlings of BBCH stage 14–16 from the greenhouse to the field in  $0.7 \text{ m} \times 0.7 \text{ m}$  planting distance [7]. The plants were left untreated for one year before the start of the experiment. Monthly average temperature, daily light integral (DLI) and precipitation were recorded (Fig. 1). The soil type of the field site is a luvisol with a clear gradient of pebble stone share (Table 1). Based on the stoniness and the higher share of the grain size fraction  $> 2 \text{ mm}$  we defined an area of “rich field soil” and “poor field soil”. Sixty subplots of  $2 \text{ m} \times 2 \text{ m}$  with an additional 1 m border zone to the neighboring plots were implemented and fertilization treatments were applied in a fully randomized design.

Additionally, an outdoor mesocosm experiment in 21 containers, each filled with 250 L of a sandy substrate (0/1 fine aggregate sand, RBS GmbH, Inden, Germany; Ca:  $0.3 \text{ g kg}^{-1}$ ; K:  $0.2 \text{ g kg}^{-1}$ ; Mg:

conditions as possible, assuming a planting density of  $100 \text{ plants m}^{-2}$  [29,30]. Seedlings of *S. hermaphrodita* of BBCH stage 13–14 were transplanted into the mesocosms in May 2014 [7]. The detailed establishment of *S. hermaphrodita* plants into the mesocosms was described earlier [27].

### 2.2. Fertilization treatments

In May 2014, 2015 and 2016 the sixty subplots of the field and the 21 mesocosms received either digestate fertilization, mineral fertilization or no fertilizer supplement as a treatment. The digestate was obtained from a commercially operating biogas plant using maize silage as feedstock (digestate dry matter mass fraction: 7.2%;  $\text{N}_{\text{total}}$ : 0.53%;  $\text{NH}_4^+$ : 0.32%; P: 0.14%; K: 0.68%; Mg 0.037%; Ca: 0.16%; S: 0.03%; organic matter: 5.3%; C:N ratio: 6; pH 8.2; all values referring to fresh weight; ADRW Naturpower GmbH & Co. KG, Titz-Ameln, Germany). NPK-fertilizer with a N:P:K-ratio similar to the digestate and a high share of ammonia was chosen to allow a comparison between the mineral and the organic digestate fertilization (NPK-fertilizer composition: N: 15% (1% nitrate; 9.5% ammonia; 4.5% isobutylidenediurea); P: 5%; K: 8%; Mg: 3%; Compo Rasendünger, Compo GmbH, Münster, Germany). Both fertilizers were calculated to provide a total nitrogen application of  $160 \text{ kg ha}^{-1}$ . We chose for this fertilization dose as it resulted in optimal plant growth in a previously published dose-response experiment for digestate fertilization of *S. hermaphrodita*, grown on the same marginal substrate used in this study [25,27].

### 2.3. Sampling and measurements

In October 2014, 2015 and 2016, the above ground biomass on both field sites and from the mesocosms was harvested and dry mass was determined after drying at  $70^{\circ}\text{C}$  to constant weight. Soil samples were taken on each plot at 0–30 cm depth and 30–40 cm distance from the plants at the time of biomass harvest and were dried to constant weight at  $30^{\circ}\text{C}$  for further analysis. N and C content of the soil and plant samples were determined by elemental analysis (VarioELcube, Elementar Analysensysteme GmbH, Langenselbold, Germany). Soil pH was determined using standard electrodes (Hanna Instruments pH 209 pH-meter, Vöhringen, Germany), using 0.01 M  $\text{CaCl}_2$  solution at  $20^{\circ}\text{C}$  as extractant.

### 2.4. Water holding capacity and soil respiration measurements

Complementary to the outdoor *S. hermaphrodita* cultivation experiments two laboratory experiments were conducted to investigate the effect of the biogas digestate on water holding capacity (WHC) and soil respiration of the marginal sandy substrate used in the mesocosm study. For WHC determination 300 g of dried sand were amended with varying digestate doses (30, 60, 90, 120, 150, 180 and  $210 \text{ g kg}^{-1}$  substrate;  $n = 4$ ) of biogas digestate, homogeneously mixed for 6 min in an end-over-end shaker and flooded with water until field capacity was reached [31]. The increasing doses were chosen to mimic multi-year application of digestate. After 24 h, when no more water was dripping out of the pots, the weight of the wet sample was determined [31]. Afterwards the wet samples were dried at  $105^{\circ}\text{C}$  to constant weight to determine the soil dry weight. Water holding capacity (WHC) was calculated by using the following equation [31]:

$$\text{Water holding capacity} = \frac{\text{total water in the wet soil (g)}}{\text{oven dry weight of total soil (g)}} \times 100$$

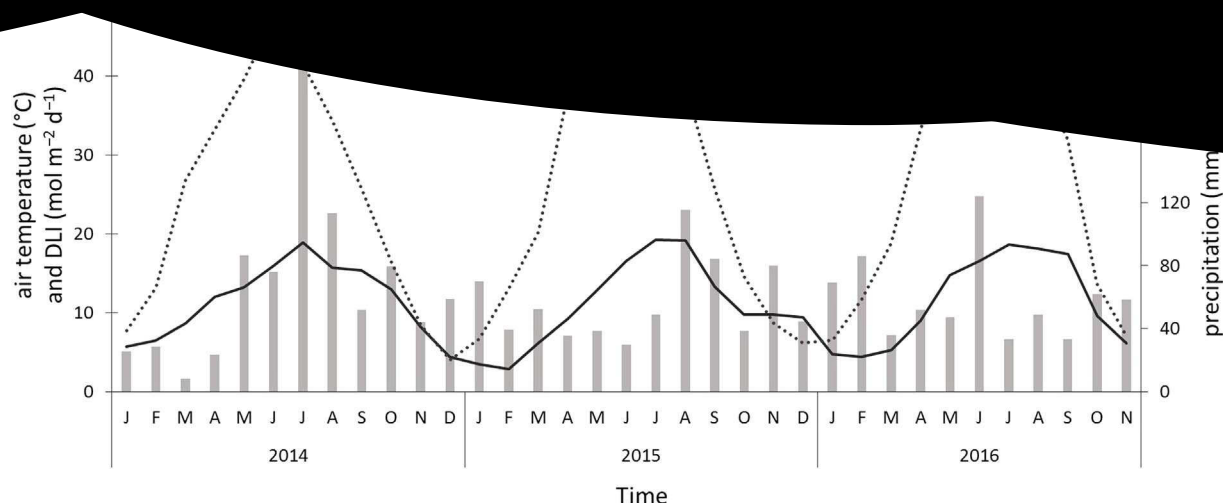


Fig. 1. Monthly mean temperature precipitation and daily light integral (DLI) values during the experimental time from 2014 to 2016 at the Research Center Jülich (50°53'47" north and 6°25'32" east; 80 m a.s.l.).

Basal soil respiration of the untreated marginal substrate and marginal substrate amended soil with two concentrations of biogas digestate (20 g kg<sup>-1</sup> and 40 g kg<sup>-1</sup>) were measured in five biological replications. For sample preparation, 30 g of homogenized samples were adjusted to 40% WHC, filled into plastic vessels and slightly compressed [32,33]. Samples were incubated at 22 °C for 120 h to stabilize respiration rates [34]. Subsequently, basal soil respiration (CO<sub>2</sub>) was measured with a Respicond VIII system (Nordgren Innovations AB, Sweden) at a constant temperature of 22 °C for 48 h [35,36].

## 2.5. Statistical analysis

The *S. hermaphrodita* cultivation experiment has a two-way factorial design with the factor fertilizer having three different levels in a completely randomized setup (control, NPK fertilization, digestate fertilization) and three different substrates (rich field soil, poor field soil and marginal sandy substrate) as second factor. The exact number of replicates per variant is given in Table 3. Statistical analysis was performed with analysis of variance (ANOVA) in R 3.0.3 (The R Foundation for Statistical Computing 2014) using the work package "Agricolae" with an *a posteriori* test as well as pairwise *t*-test [37].

## 3. Results and discussion

### 3.1. Biomass

Above-ground biomass continuously increased over the three-year duration of the experiment reaching a maximum of this three-year experiment in 2016 (Fig. 2). This corresponds well with findings of Borkowska et al. [6] who observed that above-ground biomass yields increased three to four years after planting and explained this effect with

Table 2

ANOVA results indicate that substrate quality had a greater effect on biomass yield of *Sida hermaphrodita* than fertilization. Digestate and NPK fertilization were adjusted to 160 kg N ha<sup>-1</sup>. df: Degrees of Freedom; Sum Sq: Sum of Squares.

	df	Sum Sq	Effect (%)	p-value
Soil	2	31210066	58	< 0.01
Fertilization	2	9814541	18	< 0.01
unfertilized vs. fertilized	1			< 0.01
NPK vs. Digestate	1			0.26
Fertilization x Soil	4	3004071	6	< 0.01
unfertilized vs. fertilized	2			< 0.01
NPK vs. Digestate	2			0.018
Residuals	74	9466359	18	

the perennial growth of *S. hermaphrodita*, as plants mainly invest into the establishment of a deep reaching root system in the first years [8].

Fertilization generally had a positive effect on the biomass yield on all three substrates. Highest DM yields of up to 28 t ha<sup>-1</sup> were obtained on rich field soil in the third year of the experiment, when fertilized with mineral NPK (Fig. 2). Maximum yields were clearly higher than the expected yield presented in hypothesis 1.1. In mesocosms, filled with marginal sandy substrate a maximum DM yield of 9 t ha<sup>-1</sup> was measured for plants fertilized with digestate in their third year of growth and fertilization (Fig. 2). The relative biomass stimulation of fertilized plants compared to unfertilized control plants increased with decreasing substrate quality in the following order: rich field soil: +68%; poor field soil: +71%; marginal substrate: +597%. Despite the same nutrient application, the yield differences between the two field soils can be explained by the contrasting grain size fractions, i.e. the

Table 1

The three substrates differ mainly in their grain size fraction, while pH, total organic carbon (TOC) and total nitrogen (TN) content are in a comparable range. Data show the mean of n = 3 soil samples.

	Grain size fractions (%)					pH	TOC %	TN ‰
	0–0.002 mm	0.002–0.02 mm	0.02–0.2 mm	0.02–0.2 mm	> 2 mm			
Field (rich)	16	21	58	4	2	6.2	1.0	1.0
Field (poor)	11	15	47	4	22	6.2	0.7	0.8
Sand	1	1	40	58	0	6.3	0	0

		2014			2015			2016		
rich field soil	Control	1.2 ± 0.1	1.3 ± 0.1	y	1.2 ± 0	1.4 ± 0.1	y	6.9 ± 0	x	
	Digestate	1.1 ± 0.1	3.1 ± 0.6	x	1.2 ± 0	2.8 ± 0.5	x	7.0 ± 0	x	7
	NPK	1.2 ± 0.1	1.5 ± 0.1	y	1.2 ± 0	1.6 ± 0.1	y	6.3 ± 0.1	y	7
poor field soil	Control	1.2 ± 0.1	1.4 ± 0.1	n	1.2 ± 0.1	1.4 ± 0.1	n	6.9 ± 0	n	12
	Digestate	1.3 ± 0.2	2.8 ± 0.7	m	1.2 ± 0.1	2.6 ± 0.6	m	7.1 ± 0	m	6
	NPK	0.9 ± 0.1	1.3 ± 0.1	n	0.9 ± 0.1	1.4 ± 0	n	6.2 ± 0	o	6
marginal substrate	Control	0 ± 0	0.1 ± 0	b	0.1 ± 0	0.2 ± 0	b	7.4 ± 0.1	a	7
	Digestate	0.3 ± 0.1	0.5 ± 0.7	a	0.3 ± 0.1	0.5 ± 0.1	a	6.9 ± 0.1	b	7
	NPK	0.1 ± 0	0.2 ± 0	b	0.1 ± 0	0.3 ± 0	b	6.9 ± 0.1	b	7

stoniness (Table 1). The effect of grain size fraction on soil productivity is well known and corroborates our separation of the field soils into “rich” and “poor” soil [38].

Notwithstanding the fact that we applied the same amount of N, digestate and mineral NPK fertilization differed in their growth-stimulating effects (Fig. 2, Table 2). NPK fertilization had the strongest effect on plant growth on the rich and poor field soil. A possible reason for the lower performance of digestate fertilization could be a partial immobilization of  $\text{NH}_4^+$  and immobilization of N in the biological biomass pool [39,40]. Microorganisms take up nitrogen when they have access to a carbon source like digestate [41]. The high proportion of  $\text{NH}_4^+$  in the digestate might also have resulted in losses of N via volatile  $\text{NH}_3$  [42,43].

In mesocosms, filled with marginal substrate, we observed an opposite effect, i.e. plants fertilized with digestate produced 31% higher biomass yield than those with NPK (Fig. 3). The first reason for this might be that the marginal substrate contained no traceable amounts of plant nutrients. By NPK fertilization only macro elements were applied while the digestate fertilization also contains micro nutrients [18]. However, no specific nutrient deficiency symptoms were observed

throughout all substrates and fertilization treatments over the course of three years.

Secondly, nitrate leaching out of the root horizon was found to be significant in the marginal substrate when treated with NPK-fertilizer, as described in our previous study [27]. Nitrate leaching from soil following digestate fertilization is low because nitrogen is either organically bound or in the mineral form  $\text{NH}_4^+$  which is not as mobile as nitrate [44]. During the first year of this experiment we followed nitrate concentration of the leachate and found a reduction of nitrate leaching to deeper horizons when comparing digestate to mineral NPK fertilization [27].

The third explanation for the better performance of digestate on the marginal substrate, we see in the amendment of the substrate with organic carbon. Digestate amendment increased the soil carbon content of the marginal substrate five times more than NPK fertilization [40]. The importance of the soil carbon content and its influence on soil aggregation, water holding capacity and soil fertility has been described earlier [21,22] and our results will be discussed in more detail in section 3.2. The fact that the relative advantage of digestate fertilization over mineral NPK fertilization on biomass yield gets more and more

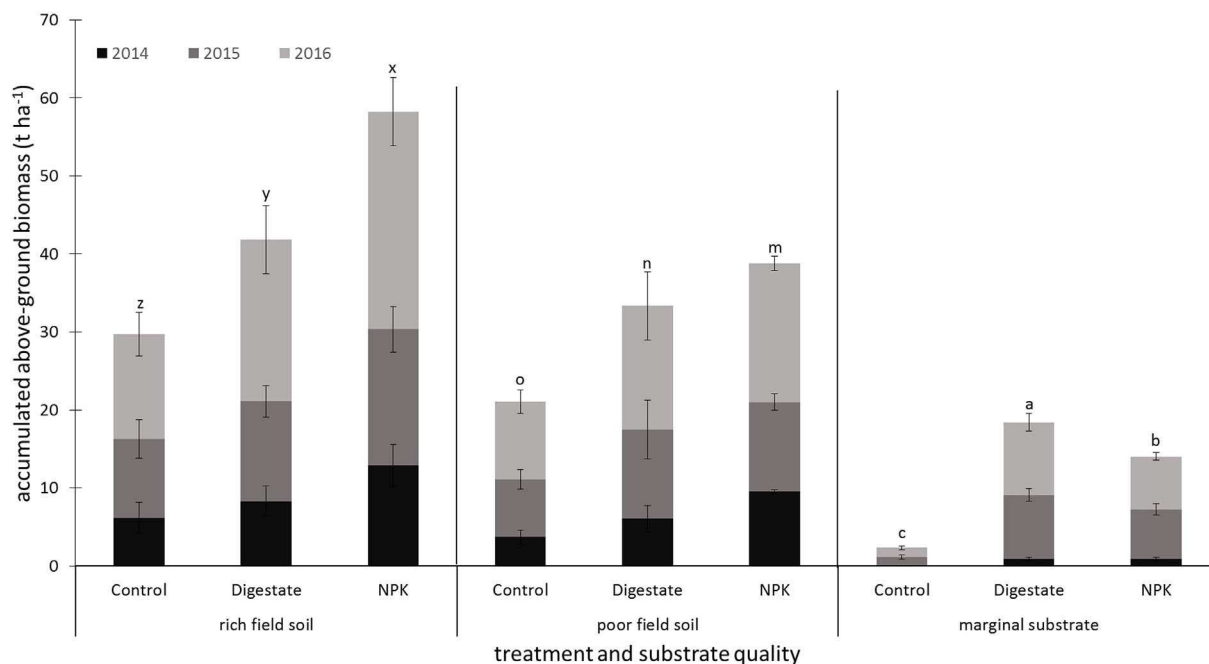
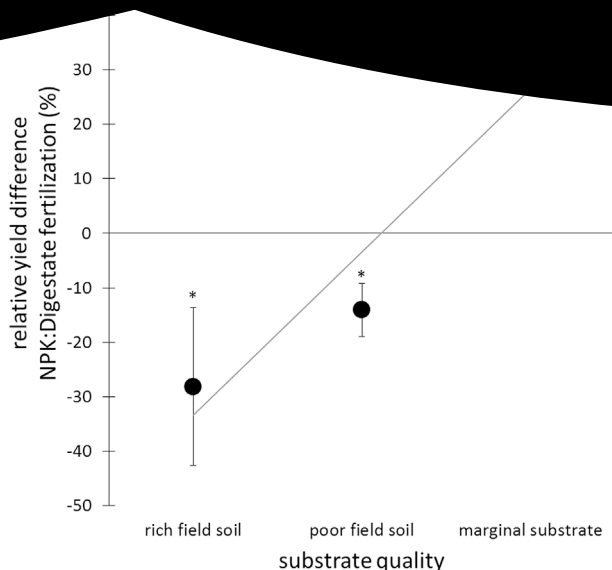
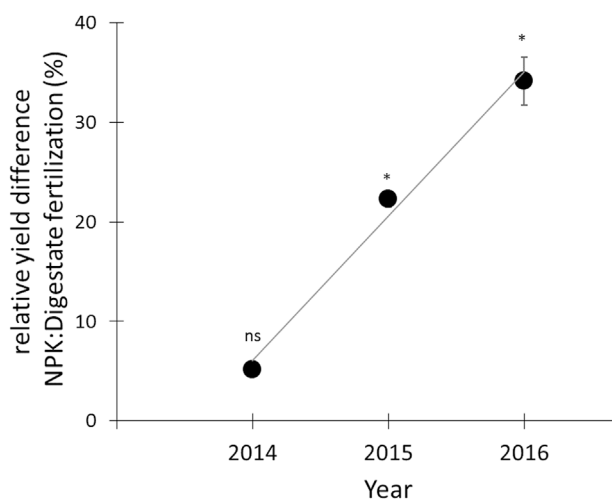


Fig. 2. The three-year cumulative biomass yield of *Sida hermaphrodita* was highly dependent on substrate type. Control: no fertilization. Digestate and NPK fertilization were adjusted to 160 kg N ha<sup>-1</sup>. Planting density 20,000 plants ha<sup>-1</sup>. Bars indicate the standard error (n = 6–26, indicated in Table 3). Within one substrate type, values of cumulative biomass with the same letter are not significantly different at p < 0.05.



**Fig. 3.** The relative yield difference between digestate and NPK fertilization indicates the digestate yield advantage on marginal substrate. Digestate and NPK fertilization were adjusted to  $160 \text{ kg N ha}^{-1}$ . Bars indicate the standard error ( $n = 6\text{--}26$ , indicated in Table 3). Data points marked with \* show a significant ( $p < 0.05$ ) yield difference between digestate and NPK fertilization.



**Fig. 4.** Relative yield difference between digestate and NPK fertilization on marginal substrate. Yield difference of digestate vs. NPK fertilization is constantly increasing over time. Digestate and NPK fertilization were adjusted to  $160 \text{ kg N ha}^{-1}$ . Bars indicate the standard error ( $n = 7$ ). Differences marked with \* are significant at  $p < 0.05$ .

pronounced over time supports the linkage to the crucial role of the soil carbon content on plant performance (Fig. 4).

In conclusion, the DM yield expectations of  $20 \text{ t ha}^{-1}$  stated in hypothesis 1.1 were exceeded for the rich field soil. Nevertheless, the strong yield reduction in the poor soil and the marginal substrate could only be partially compensated by fertilization within this three-year study. Hypothesis 1.2, foretelling better performance of NPK fertilized plants on rich substrate and better biomass yields achieved via organic fertilization on the marginal substrate, was confirmed.

### 3.2. Soil

Throughout the entire experimental period of three years, the soil carbon content in the top 30 cm increased in mesocosm and field

ping systems favors soil carbon accumulation [46]. In the field, NPK fertilization did not result in different carbon accumulation rates compared to the unfertilized control, whereas digestate fertilization resulted in an enhanced soil carbon accumulation in mesocosm and field substrates (Table 3). We conclude that the additional carbon applied via the digestate partly remained and was incorporated into the soil carbon pool [18,40].

Soil nitrogen showed the same pattern and development as the soil carbon content (Table 3). At the end of each growth season, NPK fertilization resulted in the same soil nitrogen content as found in the unfertilized control plots. As fertilization took place at the beginning of the growth period, the added nitrogen was probably already taken up by the plants or leached into deeper soil layers before samples were taken. This might be particularly the case in the poor field soil and mesocosms filled with the marginal sandy substrate due to their higher porosity and low amounts of organic carbon [27,44]. However, digestate fertilization resulted in an increase of the soil nitrogen content in all three substrates. The  $\text{NH}_4^+$  nitrogen present in the digestate gets partly immobilized by clay particles or bound to the organic fraction of the digestate [17,39]. A significant nitrogen immobilization in the case of anaerobic digestates from bark chips and organic kitchen wastes was reported already earlier [47]. Also humic compounds, that are part of the soil organic carbon are able to sequester nitrogen [48,49].

Mineral fertilization with the  $\text{NH}_4^+$ -rich fertilizer resulted in lower soil pH values compared with unfertilized control plots on all three substrates. The soil acidification effect of  $\text{NH}_4^+$  is well-known [50]. On marginal substrate digestate application resulted in a similar acidification. However, on the two field substrates digestate did not cause acidification like NPK. The high pH of the digestate itself as well as humic acids in the digestate can buffer the acidification effect of the  $\text{NH}_4^+$  in the digestate [18,23]. Unfortunately, long-term studies about the impact of digestates in soil chemical and physical properties are limited. A three year study for different digestates, performed on a loamy Retisol showed no effect on pH after three years [51]. However, Giusquiani et al. [52] found comparable results on soil pH for composts with a similar pH, and Mäder et al. [53] even measured a slight increase of pH by the application of farm yard manure. Nevertheless, these studies were performed on different soil types and did not consider perennial cultures.

The results for soil carbon content, soil nitrogen content and pH indicate the potential of the combination of perennial cropping systems with organic fertilization for soil carbon accumulation and increased soil fertility as stated previously [21,22,47]. Hornick and Parr showed that the productivity of marginal soils with stony and sandy texture was strongly increased by its amelioration with composted manure and sewage sludge both having a positive effect on soil pH, the soil water content and nutrient status of the substrate [54,55]. However, to allow a deeper understanding of the processes that lead to soil carbon accumulation and increased soil fertility a much longer timespan and soil analysis also to a sampling depth of up to 90 cm would be necessary and should encourage further research. In addition, the question to what extent the fertility and productivity of a marginal substrate can be increased would be essential to allow assessments on economic feasibility of the broader cultivation of marginal soils.

### 3.3. Water holding capacity and basal soil respiration

The complex term of soil fertility cannot be expressed merely based on plant performance. In order to get a better understanding of the interaction between organic fertilization and the marginal sandy

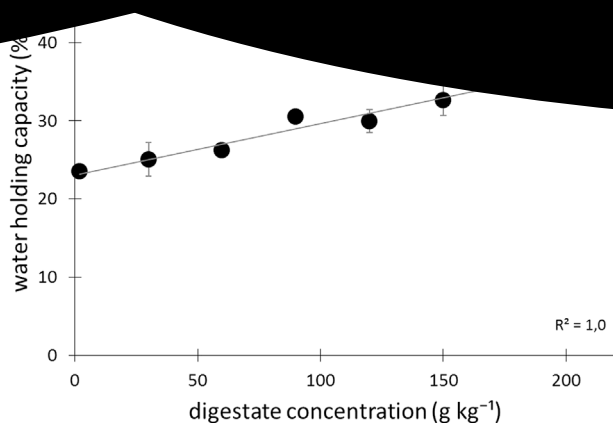


Fig. 5. The water holding capacity of the marginal substrate was positively influenced by the digestate concentration. Bars indicate the standard error ( $n = 4$ ).

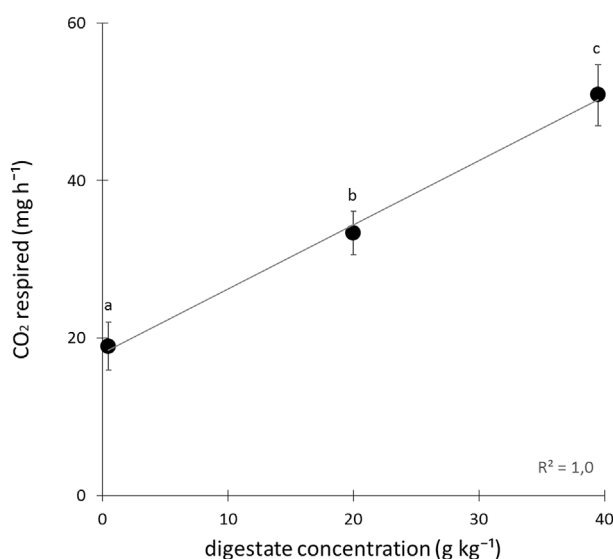


Fig. 6. Basal soil respiration of the marginal substrate increased by the addition of digestate. Bars indicate the standard error ( $n = 5$ ). Values with the same letter are not significantly different at  $p < 0.05$ .

substrate, we set up two laboratory studies focusing on water holding capacity and basal soil respiration. Here, the amendment of the marginal substrate with increasing doses of digestate showed a positive correlation for basal soil respiration and water holding capacity (Figs. 5 and 6). Albuquerque et al. [56] found similar effects on soil respiration after the amendment with digestate and argued that digestates consist of two fractions of organic matter. The first fraction is easily degradable and triggers microbial activity, whereas the second fraction is more resistant to microbial degradation, contributing to the increase of soil organic matter [40]. Furthermore, Albuquerque et al. [56] were able to positively correlate the increased soil microbial activity and soil respiration with the formation of soil aggregates, resulting in a positive effect on the water holding capacity. In line with our results, Reeves [22] describes the importance of organic fertilization to maintain or increase soil organic matter. As the biological and physical soil properties like microbial activity and water holding capacity highly depend on carbon, organic fertilization is essential for a sustainable use of soils. Based on our results, we confirmed hypothesis 2, stating a generally positive influence of organic fertilization via digestate on the soil properties of the marginal substrate, as we can prove increased soil respiration and enhanced water holding capacity, both essential

yield advantage of digestate over NPK fertilization got pronounced over the three-year experiment. Digestate fertilization increased the soil carbon content especially on the marginal substrate and thus had a beneficial effect on basal soil respiration and water holding capacity in this substrate.

Under favorable soil conditions and fertilization, i.e. “rich” field soil and NPK fertilization maximum biomass DM yields of  $28 \text{ t ha}^{-1}$  of *S. hermaphrodita* were reached. Not surprisingly, we found that with declining soil quality, the yield was reduced which could not be fully compensated by fertilization.

Even though NPK fertilization performed better on the rich soil compared to digestate fertilization, the organic fertilization is the favorable choice for the cultivation of the perennial energy crop *S. hermaphrodita* on marginal substrates. The combination of the perennial crop *S. hermaphrodita* and organic fertilization via digestate allows for an increase of the soil carbon content and an improvement of the soil fertility, resulting in an increased biomass yield over the first three years of this combined field and mesocosm experiment.

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