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Research paper

Energizing marginal soils – The establishment of the energy crop *Sida hermaphrodita* as dependent on digestate fertilization, NPK, and legume intercropping

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ABSTRACT

Growing energy crops in marginal, nutrient-deficient soils is a more sustainable alternative to conventional cultivation. The use of energy-intensive synthetic fertilizers needs to be reduced, preferably via closed nutrient loops in the biomass production cycle. In the present study based on the first growing season of a mesocosm experiment using large bins outdoors, we evaluated the potential of the energy plant *Sida hermaphrodita* to grow in a marginal sandy soil. We applied different fertilization treatments using either digestate from biogas production or a commercial mineral NPK-fertilizer. To further increase independence from synthetically produced N-fertilizers, the legume plant *Medicago sativa* was intercropped to introduce atmospherically fixed nitrogen and potentially facilitate the production of additional *S. hermaphrodita* biomass. We found digestate to be the best performing fertilizer because it produced similar yields as the NPK fertilization but minimized nitrate leaching. Legume intercropping increased the total biomass yield by more than 100% compared to *S. hermaphrodita* single cropping in the fertilized variants. However, it negatively influenced the performance of *S. hermaphrodita* in the following year. We conclude that a successful establishment of *S. hermaphrodita* for biomass production in marginal soils is possible and digestate application formed the best fertilization method when considering a range of aspects including overall yield, nitrate leaching, nitrogen fixation of *M. sativa*, and sustainability over time.

1. Introduction

In a growing bio-based economy, there is an urgent need for renewable resources. Energy crops can substitute fossil resources and help fulfill the targets for the mitigation of climate change [1]. However, food and energy crops compete for arable land, which negatively affects food security [2,3]. In the present study we investigated a novel cropping system approach for perennial energy crop production on marginal soils with the aim to contribute to more sustainable bioenergy production that aims to avoid land-use conflicts including fuel-versus-food conflicts. We use the ability of the perennial energy crop *Sida hermaphrodita* to grow on marginal sandy soils and combine it with the idea of agricultural production in closed nutrient loops by applying digestate as a fertilizer and soil amendment increasing the soil fertility. Further, we integrate intercropping of *Medicago sativa* to fix additional nitrogen to the production system and produce additional biomass at the same time. The strength of this approach is based on the combination of components that have already proven their effectiveness.

Marginal soils are rocky, sandy, or shallow with a limited reservoir of nutrients and water available to plants [4]. The European Environmental Agency (EEA) defines marginal land as low quality from an intensive agriculture viewpoint, where production barely covers cultivation costs [5]. The choice for a marginal land suitable for energy crop production needs to be made carefully because many areas with marginal soils harbor very high biodiversity and provide a large range of ecosystem functions and services [6,7]. However, the cultivation of perennial energy crops can

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have a positive influence on ecosystems and biodiversity [8]. For example, Murray et al. found a positive effect on grassland bird populations by cultivating switchgrass on former marginal cropland [9]. Besides ecosystem benefits, landscape benefits, such as the purification of air and water, generation of soil fertility, and aesthetic beauty are receiving more attention, confirming the need for novel cropping systems [10].

Sida hermaphrodita (L.) Rusby offers a promising alternative to conventional annual energy crops. *S. hermaphrodita* is a North American native prairie species from the Midwest belonging to the Malvaceae family. As a typical tall prairie forb species, it grows in sandy or rocky soils with low organic matter content and produces relatively high biomass yields in light soils with low nutrient levels [11]. It is a perennial crop that allows biomass production in a no-tillage system, conserving the soil structure, which is highly relevant for marginal soils [12]. Because *S. hermaphrodita* stores assimilates in its large rhizome, it becomes competitive after the establishment year, reducing the need for weed control [13]. Furthermore, the large root-system of this perennial crop in marginal soils allows the efficient use limited nutrient and water resources [11]. *S. hermaphrodita* is already extensively used as an energy crop in Poland, where biomass yields in arable soil are comparable to those of *Miscanthus* × *giganteus* (Giant Miscanthus) [14]. No invasive behavior of *S. hermaphrodita* has been reported to date, which can probably be explained by the low germination rates of its seeds and the low competitiveness of the seedlings [15].

In addition to perennial energy plants, legumes also have the potential to grow in marginal sandy soils. *Medicago sativa* is especially known as a high-yielding perennial legume with deep-reaching roots, enabling it to access nutrient and deep water resources. If conditions are favorable, *M. sativa* can fix up to 80 kg nitrogen ha^{-1} [16]. Intercropping of *S. hermaphrodita* and *M. sativa* potentially allows combining the high biomass productivity of *S. hermaphrodita* with the N_2-fixation and supplementary biomass production of *M. sativa*. However, the differences of the biomass of *S. hermaphrodita* and *M. sativa* because of the latter's increased protein content might require adaptations for the energy production process. Intercropping can maximize the crop production per unit area per unit time. However, the yield of the individual species in the system can be lower compared to mono-cropping. Each of the plants in an intercropping system finds its niche, which allows the efficient utilization of environmental resources. Furthermore, the combination of two crops can increase the suppression of weeds, pests, and diseases because one crop might serve as a buffer for the other. The densely closed canopy in an intercropping system helps to prevent erosion, making it less susceptible to environmental risks [17]. Another advantage of the combination of *S. hermaphrodita* and *M. sativa*, is that it provides an important ecosystem service by offering pollinators an extended flowering season [18].

Fertilization of marginal sandy soils requires special attention because the low water holding capacity has a high risk of leaching, especially for water-soluble compounds like nitrate [19,20]. Organic fertilizers, such as biogas digestate, contain a high share of organically bound nutrients and thus reduce the risk of leaching [21]. Digestate is the leftover material from biogas production by anaerobic fermentation of organic materials, such as plant biomass [22]. Using digestate as fertilizer allows the reapplication of nutrients that were removed during harvesting [23,24]. Accordingly, digestates may be able to contribute to closing the nutrient cycle and makes the cropping-system independent from energy-intensive mineral fertilizer application [25,26]. However, digestate quality strongly depends on the biogas feedstock. We focused on digestate from energy-crop mono-fermentation because it is well suited for the idea of closed nutrient loops. A study by Gissén et al. showed that fertilization with digestate compared to equivalent mineral fertilization resulted in equal yields but reduced the energy-input of the cropping system by 34% [27]. Barbosa et al. found already in a previous study that digestate was a suitable fertilizer for *S. hermaphrodita* and *M. sativa* cultivation [28]. Besides the nutrients, digestate still contains a high share of recalcitrant organic carbon, which positively affects soil fertility, soil life, and accordingly increases the yield potential [26,29]. Positive effects on soil carbon and biodiversity establish a good basis for a sustainable crop cultivation [30]. In a microcosm experiment with degraded agricultural soil, Carracciolo et al. showed that the increase of soil organic matter, related to organic fertilization increased microbial diversity, offering important ecosystem services in restoring soil quality [31].

The experiment was designed to answer the following research questions and test the following hypotheses:

Question 1: Can digestate be a suitable fertilizer for *S. hermaphrodita* production in marginal sandy soil?
Hypothesis 1: Digestate fertilization delivers equal biomass yields compared to mineral fertilization but reduces the risk of nitrate leaching.
Question 2: How does intercropping of *M. sativa* influence the growth of *S. hermaphrodita* in marginal soil?
Hypothesis 2.1: The total biomass yield increases, whereas the *S. hermaphrodita* biomass yield of intercropped *S. hermaphrodita* compared to single-cropped *S. hermaphrodita* decreases.
Hypothesis 2.2: *M. sativa* can fix nitrogen via biological N_2-fixation. Digestate fertilization results in higher fixation rates than mineral fertilization because the lower nitrogen availability will favor biological N_2-fixation.

In the present study, we focused mainly on the results of the establishment year of the experiment. Our emphasis was placed on growing a vigorous and competitive crop canopy that will guarantee fast and successful regrowth of the perennial crop in the second year after its establishment; therefore, we also monitored and evaluated the regrowth of the *S. hermaphrodita* plants in the second year.

2. Materials and methods

2.1. Plant cultivation

An outdoor mesocosm experiment was established in May 2014 at the Research Centre Jülich (Forschungszentrum Jülich GmbH, location: 50.906°N 6.410°E). *S. hermaphrodita* plants were pre-cultivated from seeds in the greenhouse (light period: 16 h; day/night temperature: 22/17 °C; humidity: 60%). Single plants of uniform size and development stage (four-leaf stage, BBCH 14) [33] were transplanted on 15 May 2014 into 250 L (0.5 m^2) bins [34] placed outdoors and filled with a sandy substrate (RBS GmbH, Inden, Germany; particle size: ≤1 mm; pH_{H2O} 6.6; WHC: 24%; no detectable amounts of N, P, K, and C), which was used as a model substrate for a marginal soil [32]. Two days after transplanting all 42 mesocosms received fertilization with 500 cm^3 0.2% Hakaphos® Green (Compo GmbH, Münster, Germany; N: 20%; P: 5%; K: 10%; Mg: 2%) guaranteeing a successful establishment of the plants in the mesocosms. Rhizobia-inoculated *M. sativa* seeds (Prunella, Mantelsaat®, Feldsaaten Freudenberger GmbH & Co. KG, Krefeld, Germany) were sown directly into half of the mesocosms with a seed-density of 4 g m^{-2} based on the thousand seed weight of Medicago (Fig 1). Besides the natural precipitation, mesocosms were irrigated manually to prevent plants from drought-stress. In drought periods, all mesocosms received an irrigation of 5 L each,

Fig. 1. Experimental Setup of mesocosms in a completely randomized design. The 250 L bins were enwrapped with white fleece-tissue to prevent the black bins from heating up in the sunlight. The picture shows the experiment in September 2014, four month after planting the *S. hermaphrodita* seedlings.

every other day. The lateral sides of the black mesocosms were wrapped with white fleece-tissue to prevent strong temperature effects in the rhizosphere and bulk soil through high solar irradiance or frost [35]. Plants were grown for 6 months until the end of the growing season in October 2014. Due to the perennial nature of *S. hermaphrodita*, the plants regrew in the subsequent vegetation period and were monitored until June 2015, allowing a comparative evaluation of the plant establishment in the various cropping systems over the two growing seasons.

2.2. Fertilization

Three weeks after the transplantation to the mesocosms, plants received the following fertilization treatments. Digestate fertilized plants received 2 L of digestate per plant and mesocosm. The digestate was obtained from a commercially operating biogas plant that employs maize silage single-fermentation (DMC: 7.2%; N: 0.53%; 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). An NPK-fertilizer with an N:P:K-ratio similar to the digestate and a high share of ammonia was chosen to allow a comparison between a mineral and an organic fertilization. NPK fertilized mesocosms received 71 g of NPK fertilizer each (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 simulate a total nitrogen application of 160 kg ha^{-1}, assuming a planting density of 15,000 *S. hermaphrodita* plants per hectare. All variants were watered after the fertilizer application to stimulate the integration into the soil and to minimize N losses via ammonia evaporation [36].

2.3. Measurements

The height of *S. hermaphrodita* plants were measured regularly at two-week intervals. Plant developmental stage was determined during the same time using the BBCH-scale of Hack et al. [33]. In order to assess the risk of leaching in the sandy substrate, leachate was collected after strong rain events and stored at 4 °C until nitrate concentration measurements were performed by ion chromatography (Dionex ICS-300; Column AS23; Eluent 0.8 mM sodium bicarbonate and 4.5 mM sodium carbonate). At the end of the growing season, the aboveground biomass was separated into *S. hermaphrodita* stems, leaves, and *M. sativa* shoots. Dry mass was determined after drying at 70 °C to constant weight to allow a calculation of the leaf and stem fraction. Additionally, soil samples were taken at 0–30 cm depth at the time of biomass harvest and dried to constant weight at 30 °C. C and N content of the soil and plant samples were determined by element analysis (VarioELcube, Elementar). Soil pH was determined using standard electrodes (Hanna Instruments pH 209 pH meter), using 1:5 distilled water extract at 20 °C.

2.4. Estimation of atmospheric N_2 fixation in M. sativa and field method for nodulation assessment

In order to estimate the N_2-fixation potential of the legume *M. sativa* on the marginal substrate, we invasively assessed nodulation of *M. sativa* in late September 2014, after 4 months of growing, following a scale-based field protocol of the British Columbia Ministry of Forestry, Canada [37]. Cores of 40 cm depth and 7 cm diameter were taken and afterwards refilled with the sandy substrate. The score took into account aboveground plant vigor (based on greenness of leaves and lack of wilting) and the number of nodules as well as nodule position, color, and appearance. The final score is then separated into three different possible categories that allow a swift assessment of nodulation efficiency: 1. effective nodulation, 2. less effective nodulation or 3. not effective nodulation, thus providing a rough indication of biological nitrogen fixation. This is a rough field method, but it allows one to swiftly and somewhat accurately assess the effectiveness of nodulation. However, it does not necessarily tell how efficient the nodules are at fixing atmospheric N_2.

For this reason, we also assessed the N derived from the

atmosphere using the natural abundance $\delta^{15}N$ method. To do this, we first measured $\delta^{15}N$ of aboveground leaf tissues of both *S. hermaphrodita* and *M. sativa* in different treatments using an element analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS; IRMS IsoPrime by Micromass UK Limited). The $\delta^{15}N$ of a sample denotes the ratio of the heavier over the lighter stable isotope of N (^{15}N over ^{14}N) in a sample in relation to a standard (atmospheric N_2) [38]:

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

R_{sample} or $R_{standard}$ is the ratio of ^{15}N over ^{14}N for sample or standard, respectively. The standard for measuring $\delta^{15}N$ is atmospheric N. Because legumes fix N_2 from the atmosphere, their $\delta^{15}N$ signal is often close to that of the standard, usually around −1‰ $\delta^{15}N$, especially when they are gaining most of their N from N_2 fixation.

We then used the $\delta^{15}N$ signal to calculate the relative share of N derived from the atmosphere (Ndfa (%)) using the method of Shearer and Kohl [39] with the equation:

$$Ndfa\ (\%) = \frac{\delta^{15}\text{N } reference\ plant - \delta^{15}N\ legume}{\delta^{15}N\ reference\ plant - B} \times 100$$

The reference plant was growing in a control treatment, receiving the very same fertilization, but not being able to fix atmospheric N_2. For the B value for legumes only relying on Ndfa, we took the lowest *M. sativa* $\delta^{15}N$ signal available in the whole experiment ($\delta^{15}N$ of −0.52) because this is a more realistic estimate to compare with the mesocosm-grown *M. sativa* plants than using a signal from *M. sativa* growing in hydroponics without N in the nutrient solution [40].

Ndfa was calculated for *M. sativa* growing in the control setup, in the digestate, and the NPK treatments.

2.5. Statistical analysis

The experiment has a two-way factorial design with the factor fertilizer having three different levels in a completely randomized setup of bins at outdoor conditions (Control, NPK addition, Digestate addition) and the presence of legume plants as a second factor (+*M. sativa* intercropping, Leg; (Control + Leg; NPK + Leg; Digestate + Leg). Seven replicates were used for each treatment. The collected leachate was analyzed in four replicates. 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 [41].

3. Results and discussion

3.1. Plant height and development

The change in height and development over time was significantly different for plants receiving NPK or digestate (Fig 2). NPK fertilized plants grew and developed faster in the first year compared to digestate-fertilized plants. One month after fertilization, we found no difference in height and development stage between digestate fertilized and unfertilized control-plants, but NPK fertilized plants were 50% taller and developed significantly more side-shoots than control-plants.

Legume intercropping did not show any significant effect on plant height and development in the establishment year but significantly reduced the plant height and development of *S. hermaphrodita* plants in the second year when fertilized with NPK.

These findings corresponded well with a greenhouse experiment with *S. hermaphrodita* in a sandy substrate that received the same fertilization treatments as reported here [32]. NPK-fertilized plants reached their maximum height eight weeks after fertilization; in contrast, digestate treated plants showed a continuous growth over ten weeks. At the end of the growing season, however, no significant difference between the height of digestate and NPK fertilized plants was found, suggesting that the differences in phenology did not lead to major differences in output by the end of the season. Furthermore, NPK-fertilized plants started flowering two weeks earlier than digestate-fertilized plants. The different nitrogen forms of both fertilizers explain the faster response of NPK fertilization compared to digestate fertilization. The NPK fertilizer contained a high share of nitrate, which is directly plant-available. Digestate contained a high share of ammonia as well as organically bound nitrogen, which first needs to be microbially mineralized before being available to the plants [21,42]. Ganmore-Neumann et al. could show delayed development of tomato plants fertilized with a high share of ammonia but found this effect especially for low soil temperatures [43]. In addition, Möller and Müller compiled a report suggesting that the high carbon content in the digestate induces soil biological activity, partially immobilizing inorganic N and thus further reducing the availability of N to the *S. hermaphrodita* plants [21].

In the second year, plants started to regrow in early April. Digestate and NPK fertilized plants developed significantly more tillers than control plants (Table 1). In general, single *S. hermaphrodita* plants grew and developed faster than in the establishment year. The fertilized variants surpassed the maximum height of the previous year already in June and again no significant difference in height and development was observed between digestate and NPK fertilization. This effect may be a long-lasting effect of the digestate on nutrient availability, as well as the storage of assimilates in the rhizome [21,42].

Galvez et al. found in a soil incubation experiment slow mineralization rates of digestate, which were negatively correlated to temperature and positively to the C/N-ratio. Given a C/N-ratio of six and the cold temperatures during the winter time, we can assume that part of N in the digestate was still organically bound over winter and became mineralized when temperatures raised in spring [42]. Legume intercropping significantly reduced the number of tillers compared to single-cropped variants. Also, legume intercropping in the second year significantly reduced the growth and development of *S. hermaphrodita*. However, this effect was not found to be significant for digestate-fertilized plants. *S. hermaphrodita* plants, intercropped with *M. sativa*, were in inter-specific competition and invested more assimilates to stem-growth, whereas singly-grown *S. hermaphrodita* plants stored more assimilates in their rhizome, allowing a fast reestablishment of these individuals in the following year [44,45]. Especially NPK fertilized *S. hermaphrodita* plants intercropped with *M. sativa* showed this effect. *S. hermaphrodita* number of tillers, development and growth are on the same level as control plants, while digestate-fertilized plants did not perform significantly different from the single-cropped plants.

For practical applications, our intercropping results of *S. hermaphrodita* with the legume *M. sativa* strongly suggest that it would be best to create a form of priority effect by sowing the *M. sativa* later then planting *S. hermaphrodita* [46]. This could avoid potential competition with the legume and increase asymmetric competition advantages for *S. hermaphrodita* while at the same time ensuring extra N input from the legume over time. A similar procedure has been suggested by Kandel et al., who tested different sowing dates of *M. sativa* into sunflowers; a later sowing of *M. sativa* reduced competition pressure and could prevent yield losses of the main

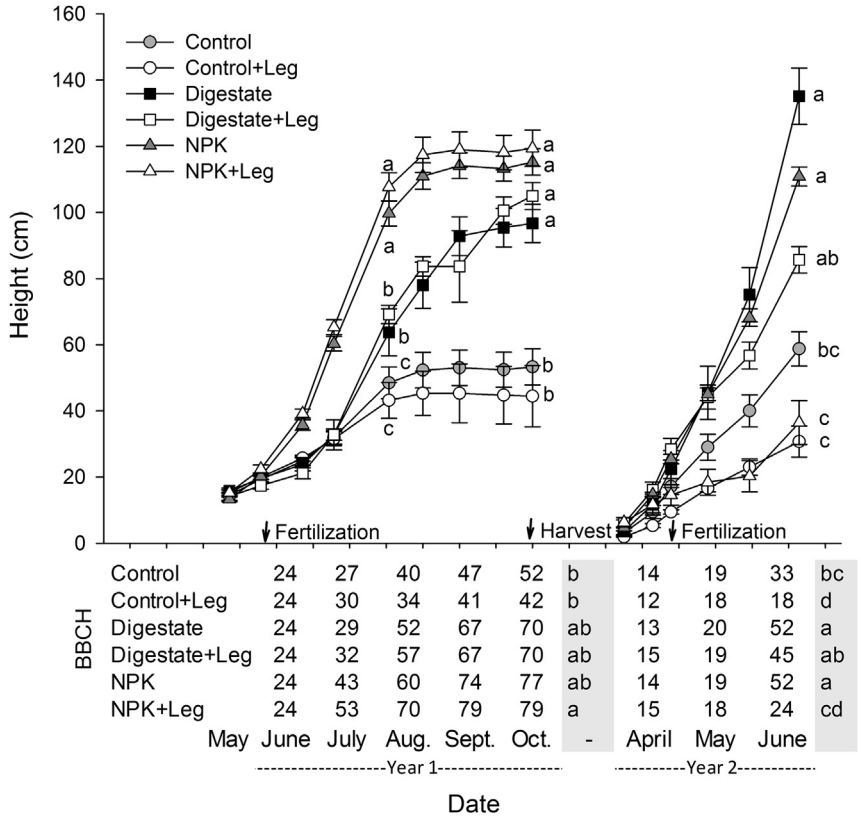

Fig. 2. Plant height and development of *S. hermaphrodita* are strongly effected by fertilization. Legume intercropping reduces plant height of *S. hermaphrodita* and delays plant development in the second year. Control: no fertilization. Digestate: 2 L mesocosm^{-1} biogas digestate. NPK: equivalent amount of NPK fertilizer. +Leg: intercropping with the legume *Medicago sativa*. Bars indicate the standard error (n = 7). Variants with the same letter are not significantly different at the 0.05 level.

crop [47]. In addition, studies of positive effects of legumes on neighbors during N facilitation have often shown that even if the biomass of the focus plant does not increase with intercropping, leaf N content often does [48].

3.2. Biomass yield

As expected, digestate and NPK application had a clear positive effect on the shoot biomass of *S. hermaphrodita* and *M. sativa* at the end of the first year. Compared to the control, the aboveground biomass of *S. hermaphrodita* was more than ten times higher for both fertilizations, and the *M. sativa* biomass over twenty times. No significant difference was found between the two fertilizers (Fig. 3). These results correspond well with findings reviewed by Möller and Müller, that digestate can be an efficient substitute for mineral fertilizers because under most conditions comparable yields can be reached [21].

Table 1
The number of tillers in the second year is reduced by legume intercropping. Tillers of *Sida hermaphrodita* in the second year. In the first year, *S. hermaphrodita* does not form tillers. Control: no fertilization. Digestate: 2 L mesocosm^{-1} biogas digestate. NPK: equivalent amount of NPK fertilizer. +Leg: intercropping with the legume *Medicago sativa*. The standard error (n = 7) is indicated by ±. Variants with the same letter are not significantly different (0.05 level).

Control	2.4 ± 0.4	b
Control + Leg	3.4 ± 1.3	b
Digestate	9.3 ± 2.1	a
Digestate + Leg	5.4 ± 0.8	ab
NPK	8.1 ± 1.7	a
NPK + Leg	3.3 ± 0.5	b

Legume intercropping reduced the shoot mass of *S. hermaphrodita* by 50% compared to the single-cropped treatments but increased the total biomass output per mesocosm by more than 100% (Fig. 3). *S. hermaphrodita* was competing with *M. sativa*,

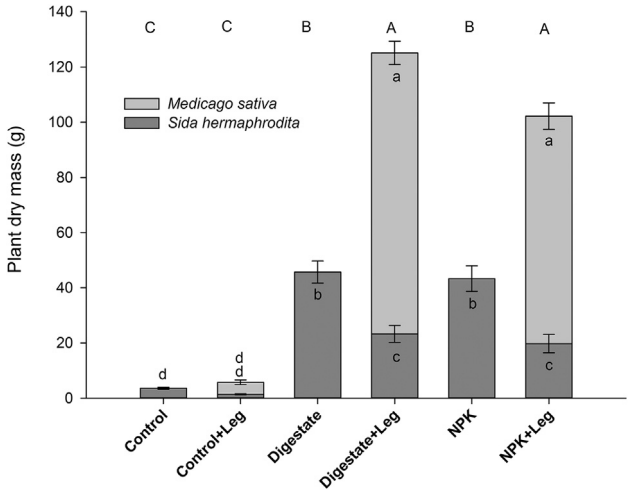

Fig. 3. Total aboveground biomass is strongly increased by intercropping *Sida hermaphrodita* with *Medicago sativa*. Control: no fertilization. Digestate: 2 L mesocosm^{-1} biogas digestate. NPK: equivalent amount of NPK fertilizer. +Leg: intercropping with the legume *M. sativa*. Bars indicate the standard error (n = 7). Variants with the same letter are not significantly different (0.05 level). Capital letters indicate differences in significance of the total above ground biomass. Small letters indicate biomass of the individual species.

resulting in lower biomass yields of *S. hermaphrodita* in the first year, corresponding with experiments intercropping *M. sativa* with grains. Zhang et al. found *M. sativa* to be the dominating and more aggressive species when intercropped with maize to fix additional nitrogen; they also observed an increased total biomass and decreased corn biomass compared to the mono-cropped control treatment [49]. For the establishment year, the yield advantage of *M. sativa* and *S. hermaphrodita* seems promising; however, the competition between the two specious negatively influenced re-sprouting in the second year and thus also might reduce overall *S. hermaphrodita* yields in the second year. However, we assume that *S. hermaphrodita* will be more competitive in the following years because it is stores parts of its assimilates in the rhizomes, allowing a fast re-sprouting in the following year. According to Borkowska et al. maximum yields of *S. hermaphrodita* can be expected following the third year after planting [44].

Legume intercropping significantly increased the fraction of stem in the aboveground biomass (SF) and reduced the leaf fraction (LF) of *M. sativa* (single-cropped: LF: 0.48, SF: 0.52; intercropped: LF: 0.25, SF: 0.75). A meta-analysis by Poorter et al. of 18 experiments shows that competition causes plants to increase their stem mass fraction while keeping similar heights. Both results correspond well with the findings of this experiment [50].

The differences in biomass yield come along with differences in the export of plant nutrients via the harvested plant biomass (Table 2). The nutrient contents of the plant material did not show differences between the different treatments. However, due to the differences in biomass yield, there are significant differences in the export of nitrogen, phosphorus, and potassium via the plant biomass. Especially the intercropping of *M. sativa* strongly increased the export of these three macronutrients.

3.3. Legume nodulation

Intriguingly, fertilization generally had a positive effect on the nodulation of the legume *M. sativa* based on the visual nodulation score (Table 3). In contrast, control plants without any fertilizer application showed ineffective nodulation. Because control plants showed reduced growth and biomass yield compared to the fertilized variants, we assume nutrient deficiency of control-plants in the sandy substrate. Experiments by Chaudhary et al. indicate that legumes suffering from nutrient deficiencies, mainly phosphorus, showed reduced nodulation [51]. However, our calculations for Ndfa show that the highest Ndfa was found in the control, followed by the digestate treatments, and very low Ndfa values were found in the NPK treatments as expected with such fertilization (Table 2). However, when looking at the absolute biological fixed nitrogen, digestate fertilized mesocosms showed by far the highest value due to the strongly increased biomass yield compared to unfertilized control plants. For NPK fertilized plants, the higher biomass yield was not able to compensate for the low share of biologically fixed nitrogen. It is well known that legumes with sufficient nitrogen supply have no preference to invest assimilates into the symbiosis with rhizobia [52]. In keeping with the Ndfa results, NPK-fertilization showed less effective nodulation in *M. sativa* than in the digestate treatment and was not significantly different from the control treatments. This could be because the addition of so many nutrients suppressed N_2 fixation initially, but with time as leaching occurred more strongly in this treatment, the legume resorted to fixing some N_2 from the atmosphere. The assessment of nodulation indicates one point in time at the end of the growing season, whereas the $\delta^{15}N$ provides an integrated signal from the entire growing season. However, NPK-treated plants showed high growth rates and high biomass yields, indicating a sufficient nitrogen supply.

Digestate-fertilized plants showed effective nodulation, indicating an intermediate plant-available nutrient pool in the soil such that the legume relied partially on N_2 from the atmosphere and the slow release of nitrogen via mineralization possibly favored nodulation [52,53]. The different influences on nodulation of NPK and digestate correspond well with findings of Nesheim et al. who also showed higher nitrogen-fixation rates of clover when fertilized with organic fertilizers rather than mineral fertilizers [54]. Furthermore, the organic carbon from the organic digestate

Table 3
Digestate fertilization favors effective nodulation and percent of N derived from the atmosphere [Ndfa(%)] of *Medicago sativa* on a sandy soil. The score follows the "Field Guide to Nodulation and Nitrogen Fixation Assessment" of the British Columbia Ministry of Forest, Canada (1991). Score 0–14: no effective nodulation. Score 15–20: less effective nodulation. Score 20–25: effective nodulation. Ndfa(%) was calculated on basis of $\delta^{15}N$-values of *M. sativa* leaves with no fertilization as a reference $\delta^{15}N$ value (control). Ndfa gives to total N fixed per mesocosm calculated on the basis of $\delta^{15}N$-measurements and measurements of the total N content of the plant biomass. Digestate: 2 L mesocosm^{-1} biogas digestate. NPK: equivalent amount of NPK fertilizer. The standard error (n = 7) is indicated by ±. Variants with the same letter are not significantly different (0.05 level).

	Nodulation score	Ndfa (%)	Ndfa (mg)
Control	11 ± 3 b	74 ± 4 a	106 ± 27 b
Digestate	20 ± 2 a	49 ± 5 b	1390 ± 83 a
NPK	16 ± 3 ab	2 ± 1 c	38 ± 3 c

Table 2
Nutrient export per mesocosm and nutrient status of the soil is effected by fertilization and legume intercropping. For plants, we show the nutrient export via the harvested biomass per mesocosm. Control: no fertilization. Digestate: 2 L mesocosm^{-1} biogas digestate. NPK: equivalent amount of NPK fertilizer. +Leg: intercropping with the legume *Medicago sativa*. Bars indicate the standard error (n = 7). Variants with the same letter are not significantly different at the 0.05 level.

			N		P		K		C		pH	
Control	Soil	mg kg^{-1}	119 ± 2	z	94 ± 1	z	272 ± 16	yz	0 ± 0	y	6.49 ± 0.08	z
	S. hermaphrodita	mg	529 ± 45	d	27 ± 3	d	599 ± 58	d				
Control + Leg	Soil	mg kg^{-1}	124 ± 2	z	102 ± 1	z	286 ± 24	yz	0 ± 0	y	6.55 ± 0.12	z
	S. hermaphrodita	mg	129 ± 20	d	7 ± 1	d	195 ± 32	d				
	M. sativa	mg	1439 ± 307	m	63 ± 14	m	795 ± 168	m				
Digestate	Soil	mg kg^{-1}	277 ± 92	z	169 ± 37	z	388 ± 16	y	692 ± 79	z	6.54 ± 0.07	z
	S. hermaphrodita	mg	6418 ± 510	b	498 ± 41	a	6533 ± 532	a				
Digestate + Leg	Soil	mg kg^{-1}	151 ± 4	z	106 ± 1	z	370 ± 6	y	591 ± 49	z	6.48 ± 0.13	z
	S. hermaphrodita	mg	2263 ± 171	c	178 ± 20	bc	2489 ± 197	bc				
	M. sativa	mg	28375 ± 1433	k	1394 ± 101	k	16087 ± 713	k				
NPK	Soil	mg kg^{-1}	117 ± 6	z	124 ± 5	z	321 ± 4	yz	453 ± 42	z	6.33 ± 0.19	z
	S. hermaphrodita	mg	9704 ± 819	a	367 ± 42	ab	5047 ± 558	ab				
NPK + Leg	Soil	mg kg^{-1}	124 ± 10	z	157 ± 18	z	186 ± 2	z	540 ± 66	z	6.36 ± 0.12	z
	S. hermaphrodita	mg	2208 ± 378	c	111 ± 19	c	1242 ± 149	c				
	M. sativa	mg	18832 ± 1107	l	770 ± 49	l	7531 ± 383	l				

fertilization with its effect on soil fertility might positively influence the nodulation. However, the effect on the soil carbon and pH content was not verifiable after the first year of this experiment but was already described (Table 2). Mekki et al. found that digestate as a soil amendment increased soil organic matter and the water retention capacity of a marginal substrate with comparable soil properties as used in our study [55].

3.4. Leaching of nitrate

Digestate fertilization significantly reduced the rate of nitrate leaching in the mesocosms compared to NPK-fertilized ones, as judged from the concentration of nitrate in the leachate (Fig. 4). However, with a nitrate peak level of 400 μg cm^{-3} eight weeks after the digestate fertilization, the concentration was eight times higher than the European threshold for drinking water of 50 μg cm^{-3}. For the NPK fertilization treatments, the measured nitrate peak value of 500 μg cm^{-3} – also eight weeks after application – was even ten times higher. From harvest time onwards, digestate-fertilized mesocosms did not exceed the threshold, whereas NPK fertilized mesocosms showed nitrate concentrations still above 100 μg cm^{-3} until the end of the measurements in late January 2015. Part of the nitrogen in the digestate remained organically bound and thus not susceptible to leaching [20,53]. Alburquerque et al. described that slow rates of microbial processes due to low temperatures in the winter reduce the mineralization of nitrogen from the digestate [19]. Di et al. assessed factors and mitigation strategies for leaching and concluded that it is essential to minimize nitrate concentrations over winter; they found organically-bound N from organic fertilization to be a potential strategy to prevent nitrate leaching [20].

Our results could be explained by the fact that *S. hermaphrodita* plants were very small at the beginning of the experiment and were not able to incorporate all available nutrients. We assume that plants will not only grow faster but also be bigger from the start in the following years, resulting in an increased uptake of more nutrients, resulting in a reduced nitrate leaching. For the establishment year of a *S. hermaphrodita* plantation in marginal soils, our results suggest to adjust the total amount of fertilizer applied and to split the fertilization into several applications of increasing dosage as described by Ingestad, who suggested adapting the nutrient supply to the current consumption of the plant [56]. Di et al. found this strategy to be an efficient mitigation of nitrate leaching because nutrients are taken up by plants before being flushed into the ground-water [20].

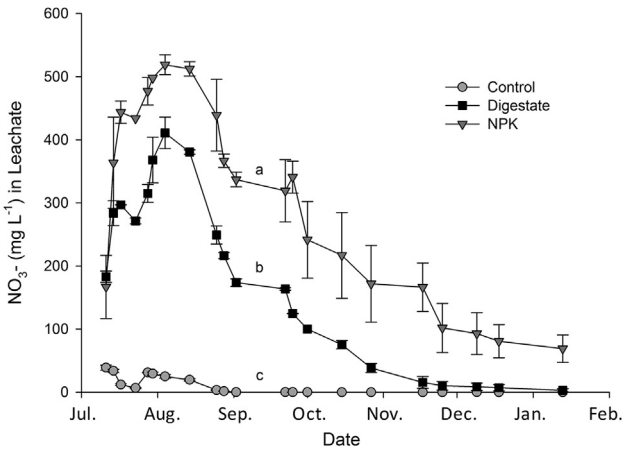

Fig. 4. Nitrate concentration of the leachate in a sandy substrate is reduced by digestate fertilization. Control: no fertilization. Digestate: 2 L $mesocosm^{-1}$ biogas digestate. NPK: equivalent amount of NPK fertilizer. Bars indicate the standard error (n = 3). Variants with the same letter are not significantly different (0.05 level).

4. Summary and conclusion

In our experiment, *S. hermaphrodita* and *M. sativa* were successfully established and grown in a marginal substrate. Fertilization with digestate or NPK increased the biomass yield in comparable ways. Legume intercropping with *M. sativa* caused clear competition with *S. hermaphrodita* plants but doubled the total biomass yield from the aboveground harvest of the community. Furthermore, *M. sativa* showed effective nodulation and fixed additional nitrogen, enriching the marginal soil with this essential plant nutrient. *M. sativa* intercropping in the second year after establishment negatively influenced the regrowth of *S. hermaphrodita* plants. We recommend sowing *M. sativa* when *S. hermaphrodita* plants are already well established to minimize the competitive effect of the legume.

As a conclusion, we find digestate to be the preferable fertilizer for *S. hermaphrodita* production in marginal soils because it resulted in a biomass yield comparable to mineral NPK-fertilization. Furthermore, it reduced the competitive effect of *M. sativa* and the nitrate concentration in the leachate while at the same time allowing an effective nodulation of *M. sativa* plants in the marginal soil, allowing a Ndfa of 40%. In contrast, NPK fertilization showed the highest nitrate losses via leaching and at the same time only showed a Ndfa of <2%. We could show that nutrients originating from plant biomass can be used as sustainable fertilizer after anaerobic digestion, following the idea of a closed nutrient loop. However, we need to find fertilization techniques that better adapted for minimizing nitrate losses via leaching in marginal soils. Both the yield developments of the different variants and the potentially beneficial long-term effects of digestate needs further investigation during the continuation of the experiment, allowing a consolidated evaluation of *S. hermaphrodita* production in marginal soils.

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