

<sup>1</sup>CEBra - Centre for Energy Technology Brandenburg e.V., Cottbus, Germany

<sup>2</sup>Brandenburg University of Technology Cottbus-Senftenberg, Chair of Soil Protection and Recultivation, Cottbus, Germany

## Effects of nitrogen and phosphate fertilization on leaf nutrient content, photosynthesis, and growth of the novel bioenergy crop *Fallopia sachalinensis* cv. 'Igniscum Candy'

Laurie Anne Koning<sup>1,2</sup>, Maik Veste<sup>1,2,\*</sup>, Dirk Freese<sup>2</sup>, Stefan Lebzien<sup>3</sup>

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### Summary

The aim of the study was to determine the effects of nitrogen and phosphate fertilization on the growth performance of the novel bioenergy crop *Fallopia sachalinensis* cv. 'Igniscum Candy' (Polygonaceae). In a controlled pot experiment various nitrogen (0, 50, 150, 300 kg N ha<sup>-1</sup>) and phosphate (20, 40, 80 kg P ha<sup>-1</sup>) fertilizer amounts were applied to measure the effect on the biomass, plant height, leaf area, and leaf nutrient (N and P) content. Furthermore, the ecophysiological processes of chlorophyll content, chlorophyll fluorescence, and gas exchange were measured. The application of nitrogen correlated positively with biomass production, while phosphate fertilization did not show a significant effect on plant growth or ecophysiological parameters. The leaf nitrogen contents were significantly correlated with the nitrogen applications, while the leaf phosphate contents did not show a correlation with the P fertilizations, but increased with the leaf nitrogen contents. A significant linear correlation between N-Tester chlorophyll meter values and chlorophyll contents as well as with leaf nitrogen contents could be determined. Under the influence of the nitrogen fertilization, net photosynthesis increased from 3.7 to 6.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The results of this experiment demonstrated that nitrogen fertilization has an overall positive correlation with leaf nitrogen content, photosynthesis, and growth of the bioenergy crop *Fallopia sachalinensis* var. Igniscum Candy.

### Introduction

Renewable energy production from biomass is of growing importance worldwide and will increase in the next decades (KALTSCHMITT et al., 2009). Biomass can be utilized in diverse manners, such as for fuel, heating, and power. In Germany, maize is currently dominating agricultural biomass production for use in biogas plants because of its high methane production (SCHITTENHELM et al., 2008; VETTER et al., 2009; FRANCK, 2012). High biomass production, biomass quality, optimal energy yield of the harvested biomass and existing harvesting technology are key factors for the selection of new plant species for bioenergy production. To avoid monospecific biomass production based on maize, other species need to also be considered. The major second generation bioenergy crops include e.g., *Miscanthus giganteus* (LEWANDOWSKI and SCHMIDT, 2006), *Panicum virgatum* (GURETZKY et al., 2011), *Sida hermaphrodita* (BORKOWSKY and MOLAS, 2012; FRANZARING et al., 2014) and *Silphium perfoliatum* (VETTER et al., 2009; GANSBERGER et al., 2014; FRANZARING et al., 2014). In this context, knotweed species (*Fallopia* spp., syn. *Reynoutria* spp.) can play important roles in the production of biomass for bioenergy in the near future (SEPPÄLÄ, 2013). The new cultivars Igniscum Basic and Igniscum Candy have been cultivated from wild forms of *Fallopia sachalinensis* hybrids for use as bioenergy crops (LEBZIEN et al., 2012). Igniscum Basic is intended for use in combined heat and power plants, while Igniscum Candy can be harvested up to two times during the growing season for biogas production. These cultivars can be alternatives to maize, adding to the portfolio of bioenergy crops. As members of a pioneer species,

the cultivars are able to grow in a wide range of habitats (ADACHI et al., 1996) and are characterized by high biomass production (PUDE and FRANKEN, 2001; VESTE et al., 2011). Knotweed species have proven themselves to achieve comparable biomass outputs to *Miscanthus*. Already in the third year of an experiment in which *Fallopia x bohémica* and *Miscanthus x giganteus* were grown under field conditions, yields were 24.2 Mg DM<sup>-1</sup> and 21.2 Mg DM ha<sup>-1</sup>, respectively (PUDE and FRANKEN, 2001). Other reported biomass production has ranged from 13.2 to 25 Mg DM ha<sup>-1</sup> depending on the particular knotweed species, soil, climate and planting density (VETTER et al., 2009; STRAŠIL and KÁRA, 2010).

It is crucial to understand plant responses to combinations of water and nutrient availability for the development of sustainable plant production systems. Nitrogen is the most common limiting factor in plant production systems. As a consequence, it is standard to add nitrogen fertilizer to a crop. But, the uptake and biomass production is crop specific e.g., switchgrass (*Panicum virgatum*) has consistently high biomass yields and relatively low nutrient removal rates, while giant reed (*Arundo donax*) with its high biomass production needs increased fertilization rates over time (KERING et al., 2012) and *Silphium perfoliatum* requires 130-160 kg N ha<sup>-1</sup> (AURBACHER et al., 2012; GANSBERGER et al., 2014). Furthermore, there are environmental concerns about the losses of nitrogen due to leaching and gaseous emissions (denitrification, ammonia volatilization and NO<sub>x</sub> emission), which are influenced by fertilization, soil type, crop species and management practices (PAUSTIAN et al., 1990; HELLEBRAND et al., 2008; CAMERON et al., 2013). The benefit of growing second generation bioenergy crops, like *Miscanthus*, *Sida*, *Silphium* and Igniscum, is their ability to store nutrients in their rhizomes over the winter months, which enables them to grow back strong in the spring (PUDE and FRANKEN, 2001; HEATON et al., 2009). This feature lowers the required nitrogen application in the early growing season. Furthermore, there can be a reduction of greenhouse gas emissions depending on the crop management (HUDIBURG et al., 2014). The other major limiting factor in plant production systems is phosphorus. Only around 15 % of the phosphate fertilizer applied to a soil is taken up by a plant from the soil solution immediately after fertilizing and the remainder relatively quickly converts to an insoluble form in the non-labile fraction (MENGEL and KIRKBY, 2001). The development of second generation bioenergy crops and optimization of their nitrogen use efficiencies will help fulfill the sustainability criteria for biomass in the production of biofuels (ERISMAN et al., 2010). Proper development of crop production systems based on specific crop and environmental quality information can lead to maximum economic returns and reductions of negative environmental consequences. The objective of our study was to investigate the relationship between nutrient supply, the ecophysiological processes and biomass production.

### Materials and methods

#### Experimental conditions for plant cultivation

One-year-old Igniscum Candy saplings grown in plastic trays were obtained from Conpower Rohstoffe GmbH and cultivated in indi-

\* Corresponding author

vidual 7 L plastic pots in the greenhouse of the Brandenburg University of Technology Cottbus-Senftenberg under semi-controlled conditions. Each pot was filled with 7 kg of silty sand with a texture of 67.7 % sand, 27.0 % silt, and 5.3 % clay. Soil nitrogen content was 0.6–0.9 g N kg<sup>-1</sup> dry soil and phosphate content was 0.3 g P kg<sup>-1</sup> dry soil. The average soil pH was 7.8.

Every pot received 200 mL of a modified Hoagland's solution (without the basic N and P content; HOAGLAND and ARNON, 1950) as a basic fertilizer. After sixty days of the establishment of the young plant in the greenhouse, the plants were cut down to allow a re-sprouting from the rhizomes; this event marked the start of the experiment. Nitrogen and phosphate fertilizers were applied to the soil surfaces. Twelve treatments with ten plants each involving every possible combination of commonly obtained nitrogen (calcium ammonium nitrate) and phosphate (superphosphate) pellet fertilizers at rates of 0, 50, 150 and 300 kg N ha<sup>-1</sup> and 20, 40 and 80 kg P ha<sup>-1</sup>, respectively, were evaluated in the experiment.

Volumetric soil moisture was measured with a frequency domain reflectometry probe (SM300, Soil Moisture Sensor Delta-T Devices, Cambridge, UK) and was kept just below the field capacity for sandy soils (14 % soil moisture). Temperature in the greenhouse ranged between 20 °C and 25 °C. Natural light was supplemented by high-pressure mercury lamps to provide photosynthetic photon flux density (PPFD) of 350–450 μmol m<sup>-2</sup> s<sup>-1</sup> at plant level for 10 hours per day. The pots were spatially sorted by treatment, while the treatments themselves were randomized in the greenhouse. Twice a week the pots were shifted in order to avoid possible spatial effects on the plants.

#### Measurements of chlorophyll contents

Leaf chlorophyll contents were assessed with a hand-held chlorophyll meter and through chemical analysis. Relative indices for the chlorophyll contents of leaves were obtained with the N-Tester chlorophyll meter from Yara (Yara International ASA, Oslo, Norway) that is based on leaf transmittance of the wavelengths 650 nm (red) and 940 nm (IR) (NETTO et al., 2005). In the standard mode the measurements of 30 leaves is averaged. Another mode offered by the device is to sample individual leaves. This individual leaf mode produces a relative index result comparable to results obtained with SPAD chlorophyll meters from Konica Minolta. Readings were taken in the middle portion of fully expanded leaves on the day of harvest. Five plants from each treatment were assessed in the standard mode while every single plant in the experiment was assessed in the individual mode. For comparison between both modes, we calculated the chlorophyll index values of the Yara-N-Tester (YNT) follow the formula:  $YNT = 15 \cdot CHL - 90$ , where YNT is the relative N-Tester index value and CHL is the chlorophyll meter value of a single leaf (Yara GmbH, Dülmen, Germany, F. Brentrup, personal communication). The same leaves that were assessed in the N-Tester individual mode were subjected to chlorophyll a and b extraction with 80 % acetone and analyzed spectrophotometrically with a Lambda 2 UV/VIS Spectrophotometer to determine total chlorophyll contents (Perkin Elmer, Norwalk, USA) after LICHTENTHALER (1987).

#### Chlorophyll fluorescence

For the measurements of *in vivo* photosynthesis on harvest day, a PAM (pulse amplitude modulation) fluorescence system (MINI-PAM Heinz Walz GmbH, Effeltrich, Germany; VESTE et al. (2000) with a 6 mm diameter standard fibre optic was used. The fibre optic was fixed at a distance of 10 mm from the leaf surface with a leaf clip holder at an angle of 60°. Standard routine programs within the PAM were used to determine effective quantum yield of photosystem II ( $\Phi_{PSII}$ ;  $(F'_m - F'_t) \cdot F'_m^{-1}$ ) in the presence of light on a photosynthesizing sample (for nomenclature of fluorescence signals used see

SCHREIBER et al., 1994; VON WILLERT et al., 1995). All chlorophyll fluorescence parameters were instantly calculated, displayed on a LCD screen and stored on an internal data logger. Photosynthetic photon flux density (PPFD) was determined with a calibrated light sensor connected with the leaf clip holder. An external halogen lamp was used to illuminate the leaf sample with  $PPFD \geq 1100 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 2 minutes prior to PAM measurements. The linear electron transport rate (ETR) for photosystem II can be calculated by multiplying the effective quantum yield of PSII ( $\Phi_{PSII}$ ) and the incident PPFD ( $ETR = \Phi_{PSII} \cdot PPFD \cdot \alpha \cdot 0.5$ ; see SCHREIBER et al., 1994; VON WILLERT et al., 1995). The leaf absorption for light ( $\alpha$ ) was set to 0.84 (VESTE et al., 2000).

#### Leaf CO<sub>2</sub> gas exchange

The gas exchange of fully expanded leaves was measured with a compact minicuvette system (CMS 400, Heinz Walz GmbH, Effeltrich, Germany; MIDGLEY et al., 1997) on the harvest day. The gas exchange measurements were conducted at the same leaves as the chlorophyll fluorescence measurements (10 leaves, 5 plants). The gas exchange measurements were carried out under ambient CO<sub>2</sub> concentrations (VESTE and HERPPICH, 1995). Illumination was set to a constant PPFD  $\geq 1100 \mu\text{mol m}^{-2} \text{s}^{-1}$  with an external halogen lamp to ensure a light-saturated photosynthesis. Air temperature in the cuvette was 25 °C and water vapor pressure deficit (VPD) of 17.4 mPa Pa<sup>-1</sup> was set with a cooling trap to correspond to the climatic conditions in the greenhouse. Changes of CO<sub>2</sub> concentration were determined with an infra-red gas analyzer (BINOS 100-4P, Rosemount, Hanau, Germany). Net CO<sub>2</sub> exchanges were calculated using the standard software DIAGAS 2.0 (Heinz Walz GmbH, Effeltrich, Germany) and CO<sub>2</sub> flux was expressed on the projected leaf area (VON WILLERT et al., 1995).

#### Biomass

The plants were harvested 173 days after the application of nitrogen and phosphate fertilizers. The fresh matter (directly after harvest) and dry weight (oven drying at 60 °C) of every plant was obtained for the biomass assessment. The harvesting procedure was carried out as follows in order to accommodate the various leaf assessments made pre- and post-harvest: I) The leaves on which individual N-Tester measurements (one leaf per plant) had been made were cut from each plant at the base of the leaves (petioles considered stem components and not leaf components) and stored in a freezer in individual plastic freezer bags. These leaves would later be subjected to a chemical analysis of chlorophyll content in the laboratory. II) The five plants from each treatment which had been used in the eco-physiological assessments, which included: N-Tester in the standard and individual modes, chlorophyll fluorescence, and CO<sub>2</sub> gas exchange were harvested in three steps. The first step was to acquire a representative mixed leaf sample for the chemical analysis of nitrogen and phosphate contents in the laboratory by cutting off ten mature leaves from halfway up the plants and packaging them in paper bags. The second step was to cut off the rest of the leaves and package them in a paper bag for the biomass assessment. The third step was to cut down the stem to just above the soil surface and package it in a paper bag for the biomass assessment. III) The other five plants in each treatment were individually harvested for the biomass assessment by cutting off the leaves and cutting down the stems, then packaging the two components separately in paper bags.

#### Analysis of nitrogen and phosphorus contents

The representative mixed leaf samples (n=10) obtained in step II of the harvesting procedure were oven-dried at 60 °C and ground up for nutrient analysis. The carbon and nitrogen contents were analyzed

with a CNS element analyzer (Vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany). The phosphorus contents of the samples were determined using a Lambda 2 UV/VIS spectrophotometer (Perkin Elmer, Norwalk, USA) following the DIN EN ISO 6878 method.

### Statistics

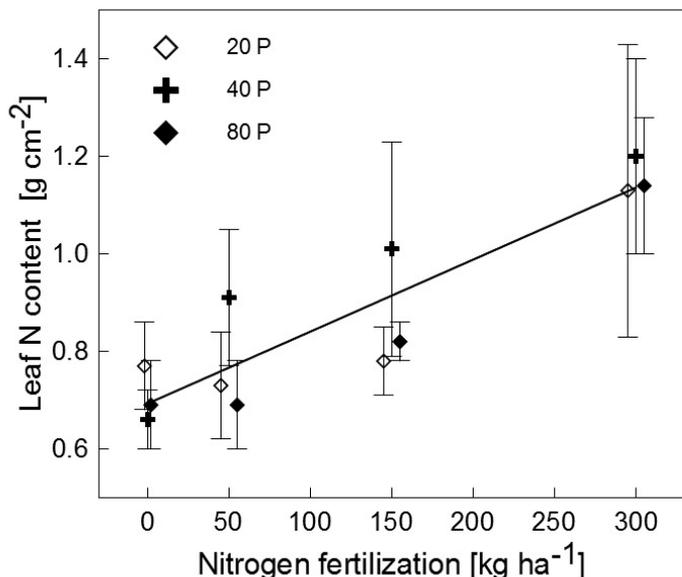
The relationships between the treatments and the independent variables were statistically assessed in the R software suite (R DEVELOPMENT CORE TEAM, 2008) and Grapher 2.0 (Golden Software Inc, Golden, USA) with linear regression analysis by applying the Pearson correlation and accepting significance at  $p < 0.05$ .

## Results and discussion

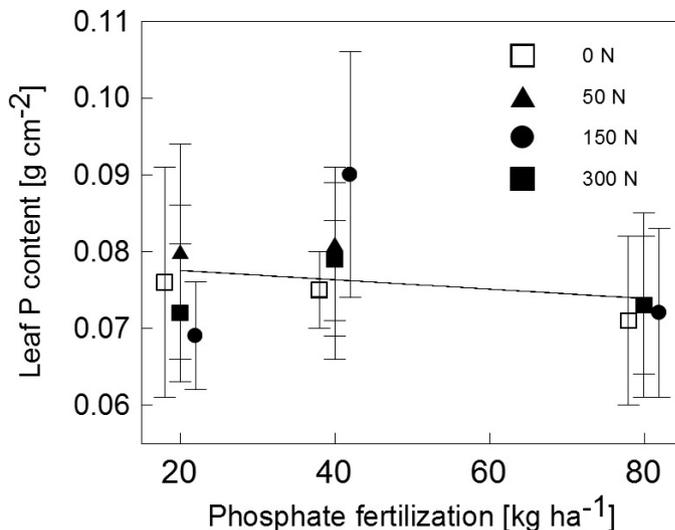
### Leaf nitrogen and phosphorus contents

In a linear regression analysis, the nitrogen contents of the leaves were significantly correlated with the nitrogen treatments in a Pearson correlation ( $R^2=0.55$ ) (Fig. 1). In contrast to this strong relationship, no correlation existed between the leaf phosphorus contents and the phosphate treatments ( $R^2=0.01$ ) (Fig. 2). A linear regression analysis of the leaf phosphorus and nitrogen contents demonstrated a weak positive trend ( $R^2=0.16$ ) (Fig. 3).

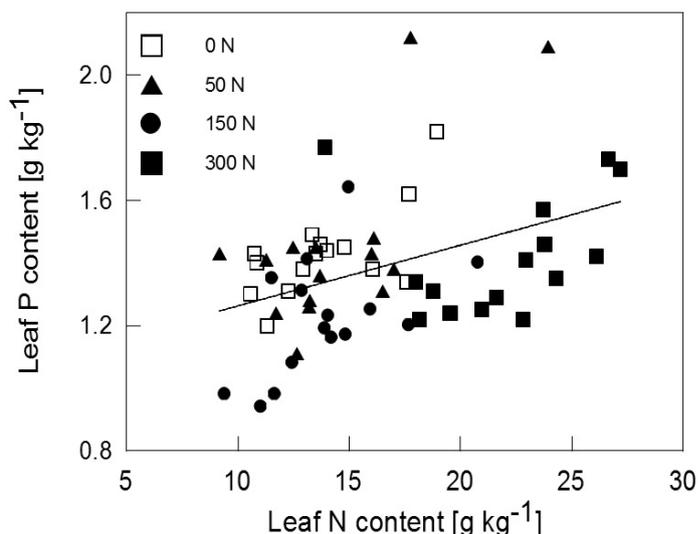
The nitrogen contents of the *Igniscum Candy* leaves in the greenhouse experiment compared well with the results of wild *Fallopia x bohemica* in Belgium, both having mean values ranging from mid 1 % to mid 2 % nitrogen (HERPIGNY et al., 2011). The nitrogen contents of *Igniscum Candy* leaves from field trial sites in Germany with various management regimes (maturely rooted crops planted in 2008 and sampled in 2010 and 2011) were higher than the greenhouse leaves, having mean values ranging from 2.8 % to 3.1 % (VESTE, unpublished data). Base nutrient levels in the respective soils may be a factor in the variance of nitrogen content (MENGEL and KIRKBY, 2001). The starting soil condition in the greenhouse experiment before fertilizer was applied averaged 0.06 - 0.09 % N. Soil analysis of the sites where *Fallopia* was found in Belgium also showed the soil to be poor in nitrogen (mean of 0.11 - 0.19 % N) and no additional fertilizer or management was applied to these wild plants before sampling took place.



**Fig. 1:** Relationship between applied nitrogen fertilizer and leaf nitrogen content at various phosphate levels (20, 40, 80 kg P ha<sup>-1</sup>). Linear regression  $y = 0.001477x + 0.692778$ ,  $R^2 = 0.3429$



**Fig. 2:** Relationship between applied phosphate fertilizer and leaf phosphorus content at various nitrogen levels (0, 50, 150, 300 kg N ha<sup>-1</sup>). Linear regression  $y = -6.071 \cdot 10^{-5}x + 0.0787$ ,  $R^2 = 2.752 \cdot 10^{-5}$



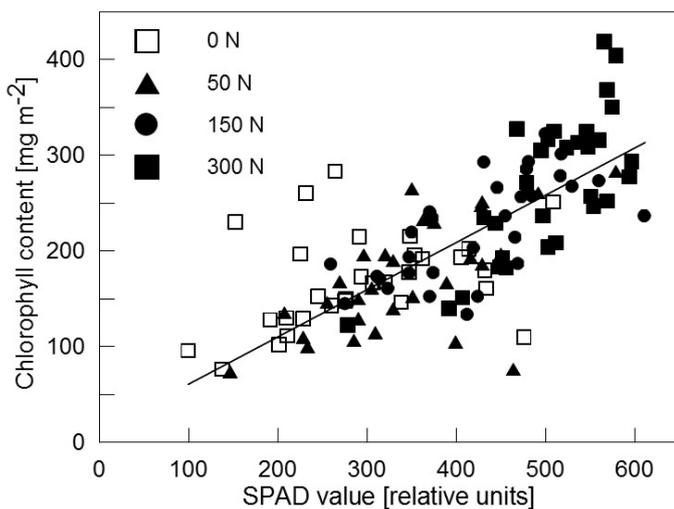
**Fig. 3:** Relationship between leaf nitrogen content and leaf phosphorus content at various nitrogen levels (0, 50, 150, 300 kg N ha<sup>-1</sup>). Linear regression  $y = 0.019396x + 1.06893$ ,  $R^2 = 0.474$

The amount of influence of the P fertilizer in this experiment is inconclusive. All three P treatments (20 P, 40 P, and 80 P) delivered similarly uneventful results for nutrient contents and biomass. A plot of the P content in the leaves against the N treatments showed no significant trends ( $R^2=0.01$  and  $R^2=0.07$ , respectively), suggesting that nutrient uptake was not influenced by the P fertilization rates. A plot of dry matter against the P treatments also produced a lack of trend ( $R^2=0.00$ ) (data not shown). Phosphorus uptake is not well understood (SCHACHTMAN et al., 1998). An investigation into the availability of phosphorus in the soil or a biological analysis of the phosphorus distribution throughout the entirety of the plants (e.g. VENEKLAAS et al., 2012) may have shed more light on the phosphorus uptake by *Igniscum*.

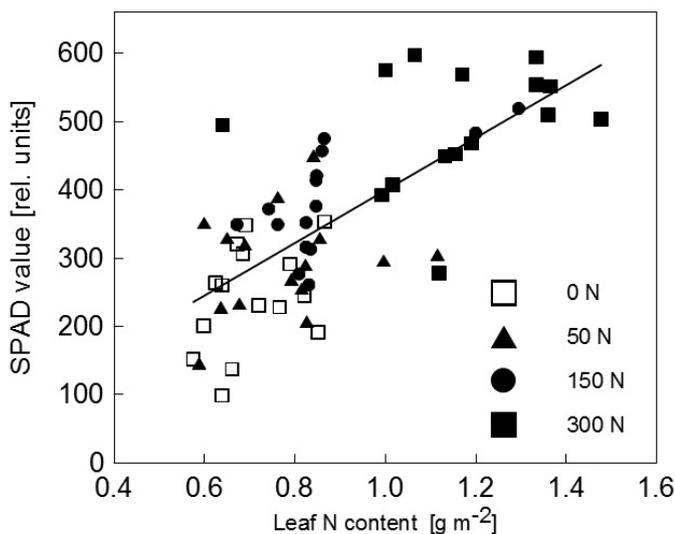
### Chlorophyll content

Previous studies have shown that the chlorophyll contents of leaves correlates strongly with the nitrogen contents of leaves (GIANQUINTO

et al., 2004; SAMBORSKI, 2009) and based on this relationship, handheld devices measuring the chlorophyll in living plants can reflect the nitrogen status and physiological activity of plants (BULLOCK et al., 1998; NETTO et al., 2005). The relative indices measured by the N-Tester were in significant linear correlation to the chlorophyll contents of the leaves ( $R^2=0.65$ ) (Fig. 4) and the nitrogen contents of the leaves ( $R^2=0.52$ ) (Fig. 5). As expected, a significant linear correlation also existed between the chlorophyll contents of the leaves and the nitrogen contents of the leaves ( $R^2=0.42$ ) (not shown). The theory behind the N-Tester was supported by the current research through strong positive trends in the relationships between the chlorophyll and nitrogen contents as well as the N-Tester results and nitrogen contents.



**Fig. 4:** Relationship between Yara N-Tester values and chlorophyll content (linear regression  $y = 0.4924x + 11.871$ ,  $R^2 = 0.645$ ) at nitrogen levels (0, 50, 150, 300 kg N ha<sup>-1</sup>).

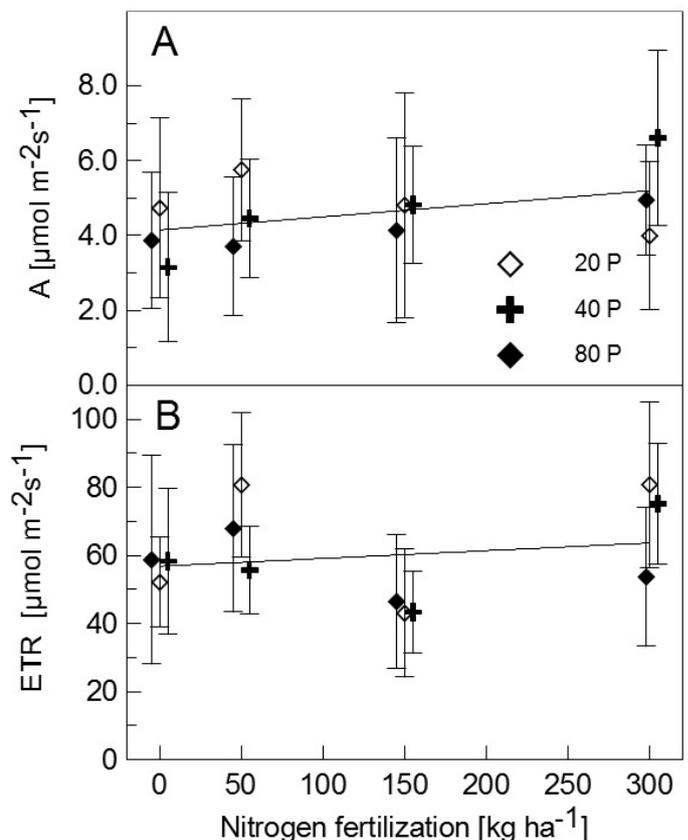


**Fig. 5:** Relationship between leaf nitrogen content and Yara N-Tester value (linear regression  $y = 384.918x + 14.2766$ ,  $R^2 = 0.518$ ) at various nitrogen levels (0, 50, 150, 300 kg N ha<sup>-1</sup>).

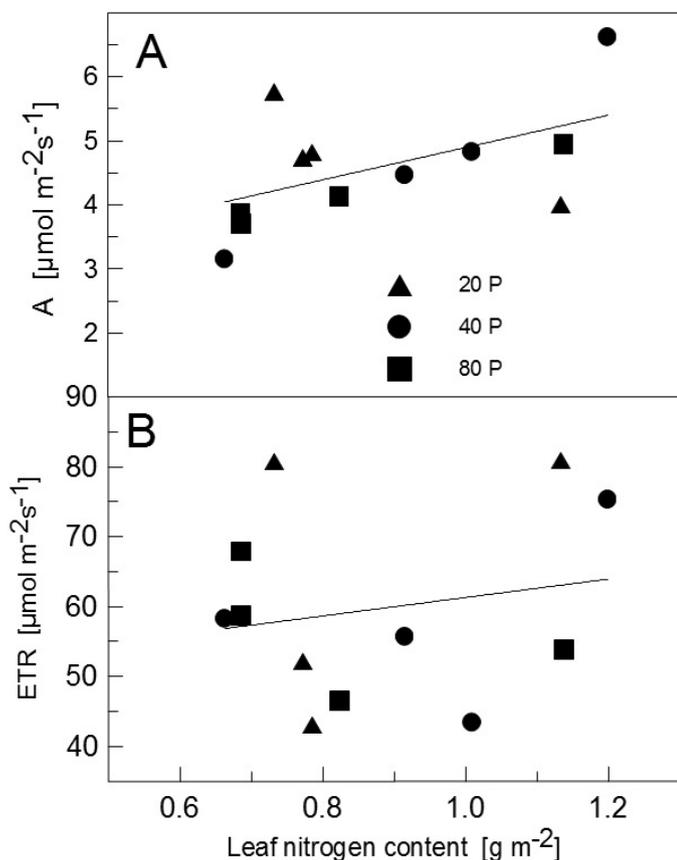
### Leaf photosynthesis

The mean net photosynthesis rates varied between 3.7 and 6.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and increased ( $R^2=0.251$ ) with increasing nitrogen application (Fig. 6a), while the electron transport rates showed no changes

between the different nitrogen treatments (Fig. 6b). Even though photosynthesis depends on phosphate containing compounds and phosphate efficiency should influence photosynthetic activity (VENEKLAAS et al., 2012), we did not find any relation between the photosynthesis parameters and the phosphate fertilization. However,  $\text{CO}_2$  exchange rates and electron transport rates showed a high variance within each treatment. The measured net photosynthesis of *Fallopia* is lower compared to other perennial bioenergy crops or highly productive annual crops. MANTOVANI et al. (2014) measured mean  $\text{CO}_2$  exchange rates of 7.5 to 9.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for *Igniscum* in a lysimeter experiment. For the perennial *Sida hermaphrodita*, which is considered a highly productive bioenergy crop, mean net photosynthesis rates from 6 - 17  $\mu\text{mol m}^{-2} \text{s}^{-1}$  were measured (FRANZARING et al., 2014; VESTE et al., 2014), while annual crops like sunflowers or tomatoes reach net photosynthesis rates of 12 - 24  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 18 - 26  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (BRECKLE et al., 2003). In general, the photosynthesis and related physiological processes are directly linked to the leaf nitrogen content (EVANS, 1983; SAGE et al., 1987; LAWLOR et al., 2001). In our experiment a linear relationship between the leaf N content and the net  $\text{CO}_2$  exchange could be shown (Fig. 7a), while the electron transport rate showed no correlation with the leaf N content (Fig. 7b). However, a clear linear influence of nitrogen leaf content on photosynthesis could be observed under various field conditions in Germany (VESTE et al., 2011). Furthermore, nitrogen also alters leaf morphology (LAWLOR, 2001). Since  $\text{CO}_2$  exchange rates are area related, leaf morphological changes in response to nitrogen needs to be taken into account to understand the plant  $\text{CO}_2$  uptake. Hereby, the leaf area demonstrated a strong positive trend over the increasing N treatments (Fig. 8c) and increased total carbon uptake. It needs to be considered that nutrient deficiency



**Fig. 6:** Influence of the applied nitrogen fertilizer on net  $\text{CO}_2$  exchange (A) and electron transport rate (B). (A) Linear regression  $y = 0.00216963x + 1.133749$ ,  $R^2 = 0.2013$ , (B) Linear regression  $y = 0.022849x + 56.83$ ,  $R^2 = 0.004$ .

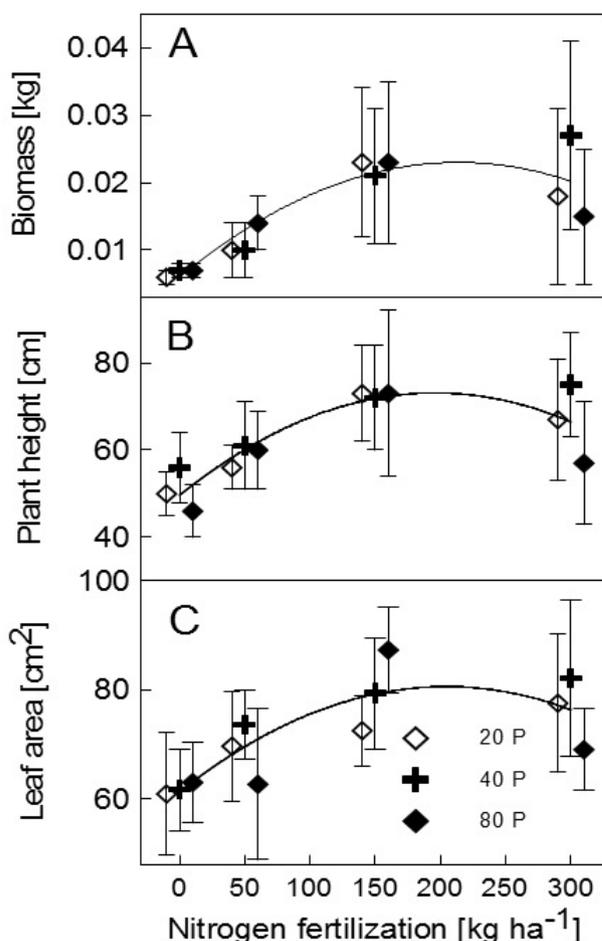


**Fig. 7:** Relationship between leaf nitrogen content and net CO<sub>2</sub> exchange (A) and electron transport rate (B) at various phosphate levels (20, 40, 80 kg P ha<sup>-1</sup>). (A) Linear regression  $y = 2.518x + 2.378$ ,  $R^2 = 0.251$ , (B) Linear regression  $y = 13.173x + 48.117$ ,  $R^2 = 0.134$ .

might also affect the carbon source-sink interrelation. Understanding the carbon-sink interrelation as well as carbon allocation is important for understanding the growth performance of plants in relation to their photosynthesis (KÖRNER, 2013; FATICHI et al., 2014). In the case of the studied *Fallopia*, the formation of the underground rhizome to store carbon and nutrients during the establishment of young plants is such an example of carbon and nutrient flows into different sink organs. However, in mature established plants the interrelation between aboveground and belowground organs is important to understand biomass production under different nutrient supply and harvest regimes (ADACHI et al., 1996; LI et al., 1998; ARAVINDHAKSHAN et al., 2011) and needs further investigations in herbaceous perennial bioenergy plants.

### Biomass production

The dry weight, plant height, and leaf area (Fig. 8) all had quadratic relationships with the nitrogen treatments ( $R^2=0.32, 0.34, 0.32$ , respectively). The peaks of the parabolic curves, or the point of optimum yield for dry weight, were reached around 170 kg N ha<sup>-1</sup>. A complete lack of correlation ( $R^2=0.00$ ) existed between the dry weight and phosphate treatments (not shown). Our experiment showed that increasing nitrogen fertilization to young knotweed plants leads to higher biomass yields, as shown previously by SCHMITT (1994). The strongest effect of the nitrogen supply was from 100 to 150 kg N ha<sup>-1</sup>, which could also be shown in a lysimeter experiment (MANTOVANI et al., 2014) and is in the same range as observed for *Silphium perfoliatum* (GANSBERGER et al., 2014). The positive effects of the nitrogen fertilization on biomass, plant height and leaf area showed



**Fig. 8:** Influence of the applied nitrogen fertilizer on aboveground biomass (A), plant height (B) and total leaf area (C) at various phosphate levels (20, 40, 80 kg P ha<sup>-1</sup>). (A) Biomass ( $n = 10$ ,  $R^2 = 0.32$ ), (B) plant height ( $n = 10$ ,  $R^2 = 0.34$ ), and (C) leaf area ( $n = 5$ ,  $R^2 = 0.32$ ).

a general decline between the 150 and 300 kg N ha<sup>-1</sup> treatments, possibly due to nitrogen uptake entering the zone of luxury nutrient consumption (WESTERMAN, 1990). PUDE and FRANKEN (2001) observed an increase in biomass production with up to 200 kg N ha<sup>-1</sup>, while on agricultural fields of the Soester Börde (Northrhine Westfalia, Germany) the growth response to nitrogen fertilization was indifferent (VESTE et al., 2011). As shown in Fig. 8c, the leaf area of the *Igniscum* plants in this experiment ranged between 50 and 100 cm<sup>2</sup>. Consequently, plants in the current experiment did not reach their full leaf area potentials between 150 and 350 cm<sup>2</sup> (HERPIGNY et al., 2011) or 300 cm<sup>2</sup> (BAILEY and BIMOVA, 2009) as described for *F. x bohemica*. Just as the plants in the greenhouse did not reach as high of nitrogen contents nor produce as high of relative indices with the N-tester as the plants in field trials, the small size of the leaves could likely be contributed to the more limited greenhouse conditions as compared to the outdoor sites (WEIH and NORDH, 2005).

### Conclusions

The results of this experiment demonstrated that nitrogen fertilization has an overall positive correlation with leaf nitrogen content, photosynthesis, and growth of the bioenergy crop *Fallopia sachalinensis* var. *Igniscum* Candy. The existence of significant trends in the influence of phosphate fertilizer on *Igniscum* Candy could not be demonstrated and requires further investigation.

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## References

- ADACHI, N., TERASHIMA, I., TAKAHASHI, M., 1996: Nitrogen translocation via rhizome systems in monoclonal stands of *Reynoutria japonica* in an oligotrophic desert on Mt Fuji: Field experiments. *Ecological Research* 11 (2), 175-186.
- ARAVINDHAKSHAN, S.C., EPLIN, F.M., TALIAFERRO, C.M., 2011: Switchgrass, Bermudagrass, Flaccidgrass, and Lovegrass biomass yield response to nitrogen for single and double harvest. *Biomass Bioenerg.* 35, 308-319.
- AURBACHER, J., BENKE, M., FORMOWITZ, B., GLAUERT, T., HEIERMANN, M., HERRMANN, C., IDLER, C., KORNAZ, P., NEHRING, A., RIECKMANN, C., RIECKMANN, G., REUS, D., VETTER, A., VOLLRATH, B., WILKEN, F., WILLMS, M., 2012: Energiepflanzen für Biogasanlagen. Regionalbroschüre Niedersachsen. Fachagentur Nachwachsende Rohstoffe e.V. (FNR), Gülzow-Prüzen.
- BORKOWSKY, H., MOLAS, R., 2012: Two extremely different crops, Salix and Sida, as source for renewable bioenergy. *Biomass Bioenerg.* 36, 234-240.
- BULLOCK, D.G., ANDERSON, D.S., 1998: Evaluation of the Minolta SPAD-502 chlorophyll meter for nitrogen management in corn. *J. Plant Nutr.* 21 (4), 741-755.
- CAMERON, K.C., DI, H.J., MOIR, J.L., 2013: Nitrogen losses from the soil/plant system: a review. *Ann. Appl. Biol.* 162, 145-173. doi: 10.1111/aab.12014
- ERISMAN, J., GRINSVEN, H., LEIP, A., MOSIER, A., BLEEKER, A., 2010: Nitrogen and biofuels: an overview of the current state of knowledge. *Nutr. Cycl. Agroecosys.* 86, 211-223.
- FATICHI, S., LEUZINGER, S., KÖRNER, C., 2014: Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. *New Phytol.* 201, 1086-1095.
- FRANCK, E., 2012: Maisanbau in Niedersachsen. Standort 36, 194-198.
- FRANZARING, J., SCHMID, I., BÄUERLE, L., GENSHEIMER, G., FANGMEIER, A., 2014: Investigations on plant functional traits, epidermal structures and the ecophysiology of the novel bioenergy species *Sida hermaphrodita* Rusby and *Silphium perfoliatum* L. *J. Appl. Bot. Food Qual.* 87, 36-45.
- GANSBERGER, M., MONTGOMERY, L.F.R., LIEBHARD, P., 2014: Botanical characteristics, crop management and potential of *Silphium perfoliatum* L. as a renewable resource for biogas production: A review. *Industrial Crops and Products*, <http://dx.doi.org/10.1016/j.indcrop.2014.09.047>.
- GIANQUINTO, G., GOFFART, J.P., OLIVIER, M., VOS, J., MACKERRON, D.K.L., 2004: The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. *Potato Res.* 47, 35-80.
- GURETZKY, J., BIERMACHER, J., COOK, B., KIERING, M., MOSALI, J., 2011: Switchgrass for forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient composition. *Plant Soil* 339, 69-81.
- HEATON, E.A., DOHLEMAN, F.G., LONG, S.P., 2008: Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Glob. Change Biol.* 14, 2000-2014.
- HEATON, E.A., DOHLEMAN, F.G., LONG, S.P., 2009: Seasonal nitrogen dynamics of *Miscanthus x giganteus* and *Panicum virgatum*. *GCB Bioenergy* 1, 297-307.
- HELLEBRAND, H.J., SCHOLZ, V., KERN, J., 2008: Nitrogen conversion and nitrous oxide hot spots in energy crop cultivation. *Res. Agri. Eng.* 54, 58-67.
- HOAGLAND, D.R., ARNON, D.I., 1950: The water-culture method for growing plants without soil. California Agricultural Experiment Station, Circular 347, University of California, 1-32.
- HUDIBURG, T.W., DAVIS, S.C., PARTON, W., DELUCIA, E.H., 2014: Bioenergy crop greenhouse gas mitigation potential under a range of management practices. *GCB Bioenergy*. doi: 10.1111/gcbb.12152
- KALTSCHMITT, M., HARTMANN, H., HOFBAUER H., 2009: Energie aus Biomasse. Grundlagen, Techniken und Verfahren. Springer, Berlin, Heidelberg, New York.
- KÖRNER, C., 2013: Growth controls photosynthesis – mostly. *Nov. Acta Leopoldina* NF 114, Nr. 391, 273-283.
- LAWLOR, D.W., 2001: Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J. Exp. Bot.* 53, 773-787.
- LAWLOR, D.W., LEMAIRE, G., GASTAL, F., 2001: Nitrogen, plant growth and crop yield. In: Lea, P.J., Morot-Gaudry, J.-F. (eds.), *Plant nitrogen*, 343-367. Springer-Verlag, Berlin.
- LEBZINI, S., VESTE, M., FECHNER, H., KONING, L., MANTOVANI, D., FREESE, D., 2012: The Giant Knotweed (*Fallopia sachalinensis* var. *Igniscum*) as a new plant resource for biomass production for bioenergy. *Geophys. Res. Abstr.* 14, EGU2012-6060.
- LEWANDOWSKI, I., BÖHMEL, C., VETTER, A., HARTMANN, H., 2009: Landwirtschaftlich produzierte Lignocellulosepflanzen. In: Kaltschmidt, M., Hartmann, H., Hofbauer, H. (eds.), *Energie aus Biomasse. Grundlagen, Techniken und Verfahren*, 88-108. Springer, Heidelberg.
- LEWANDOWSKI, I., CLIFTON-BROWN, J.C., SCURLOCK, J.M.O., HUISMAN, W., 2000: *Miscanthus*: European experience with a novel energy crop. *Biomass Bioenerg.* 19, 209-227.
- LI, R., WERGER, M.J.A., DURING, H.J., ZHONG, Z.C., 1998: Carbon and nutrient dynamics in relation to growth rhythm in the giant bamboo *Phyllostachys pubescens*. *Plant Soils* 201, 113-123.
- LICHTENTHALER, H.K., 1987: Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 48, 350-382.
- MANTOVANI, D., VESTE, M., GYPSER, S., HALKE, C., KONING, L., FREESE, D., LEBZINI, S., 2014: Transpiration and biomass production of the bioenergy crop Giant Knotweed *Igniscum* under various supplies of water and nutrients. *Journal of Hydrology and Hydromechanics* 62, 316-323.
- MENGEL, K., KIRKBY, E., 2001: Principle's of plant nutrition. Dordrecht: Kluwer Academic Publishers.
- MIDGLEY, G., VESTE, M., VON WILLERT, D.J., DAVIS, G.W., STEINBERG, M., POWRIE, L.W., 1997: Comparative field performance of three different gas exchange systems. *Bothalia* 27 (1), 83-89.
- NETTO, A.L., CAMPOSTRINI, E., GONCALVES DE OLIVEIRA, J., BRESSAN-SMITH, R.E., 2005: Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Sci. Horti.* 104 (1), 199-209.
- PAUSTIAN, K., ANDRÉN, O., CLARHOLM, M., HANSSON, A.-C., JOHANSSON, G., LAGERLOF, J., LINDBERG, T., PETTERSSON, R., SOHLENIUS, B., 1990: Carbon and nitrogen budgets of four agro-ecosystems with annual and perennial crops, with and without N fertilization. *J. Appl. Ecol.* 27 (1), 60-84.
- PUDE, R., FRANKEN, H., 2001: *Reynoutria bohemica* – eine Alternative zu *Miscanthus x giganteus*? *Bodenkultur* 52 (1), 19-27.
- R DEVELOPMENT CORE TEAM, 2008: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- SAGE, R.F., PEARCY, R.W., SEEMANN J.R., 1987: The nitrogen use efficiency of C<sub>3</sub> and C<sub>4</sub> plants. I. Leaf nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol.* 84, 954-958.
- SEPPÄLÄ, M., ANTTI, L., JUKKA, R., 2013: Screening of novel plants for bio-gas production in northern conditions. *Bioresource Technol.* 139, 355-362.
- SCHACHTMAN, D.P. REID, R.J., AYLING, S.M., 1998: Phosphorus uptake by

- plants: from soil to cell. *Plant Physiol.* 116, 447-453.
- SCHITTENHELM, S., 2008: Chemical composition and methane yield of maize hybrids with contrasting maturity. *Eur. J. Agron.* 29, 72-79.
- SCHMITT, A., 1994: Qualitätssicherung von feldmäßig angebautem Pflanzenmaterial aus *Reynoutria sachaliensis* unter Berücksichtigung der resistenzinduzierenden Eigenschaften. Abschlussbericht zum Forschungsauftrag 91-NR 002.
- SCHREIBER, U., BILGER, W., NEUBAUER, C., 1994: Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D., Caldwell, M.M. (eds.), *Ecophysiology of Photosynthesis*, Ecological Studies 100, 49-70. Springer, New York.
- STRAŠIL, Z., KARA, J., 2010: Study of knotweed (*Reynoutria*) as possible phytomass resource for energy and industrial utilization. *Res. Agric. Eng.* 56 (3), 85-91.
- VENEKLAAS, E.J., LAMBERS, H., BRAGG, J., FINNEGAN, P.M., LOVELOCK, C.E., PLAXTON, W.C., PRICE, C.A., SCHEIBLE, W.-R., SHANE, M.W., WHITE, P.J., RAVEN, J.A., 2012: Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist* 195, 306-320.
- VESTE, M., HERPPICH, W., 1995: Influence of diurnal and seasonal fluctuations in the atmospheric CO<sub>2</sub> concentration on the photosynthesis of *Populus tremula*. *Photosynthetica* 31 (3), 371-378.
- VESTE, M., MANTOVANI, D., KONING, L., LEBZIEN, S., FREESE, D., 2011: Improving nutrient and water use efficiency of Igniscum – a new bioenergy crop. *Berichte der Deutschen Bodenkundlichen Gesellschaft*. DBG, 4p. <http://eprints.dbges.de/739/>
- VESTE, M., BEN-GAL, A., SHANI, U., 2000: Impact of thermal stress and high vpd on gas exchange and chlorophyll fluorescence of *Citrus grandis* under desert conditions. *Acta Hort.* 531, 143-149.
- VESTE, M., QUINKENSTEIN, A., FREESE, D., 2014: BioSida – Anbau von Sida als neue Kultur für Bioenergie und zur Inwertsetzung degradierter Standorte. Arbeitsgemeinschaft industrielle Forschung, Report, Cottbus, 1-35.
- VETTER, A., HEIERMANN, M., TOEWS, T., 2009: Anbausysteme für Energiepflanzen; DLG Verlag Frankfurt/Main.
- VON WILLERT, D.J., HERPPICH, W.B., MATYSEK, R., 1995: Experimentelle Pflanzenökologie. Grundlagen und Anwendungen. Thieme Verlag, Stuttgart.
- WEILAND, P., 2010: Biogas production: current state and perspectives. *Appl. Microbiol. Biot.* 85, 849-860.
- WESTERMAN, R., 1990: Soil testing and plant analysis. Madison, Wisconsin, USA: Soil Science Society of America, Inc.

#### Addresses of the authors:

Laurie A. Koning, Maik Veste, CEBra – Centre for Energy Technology Brandenburg e.V., Friedlieb-Runge-Straße 3, 03046 Cottbus, Germany; Brandenburg University of Technology Cottbus-Senftenberg, Chair of Soil Protection and Recultivation, Konrad-Wachsmann-Allee 6, 03046 Cottbus, Germany  
 Dirk Freese, Brandenburg University of Technology Cottbus-Senftenberg, Chair of Soil Protection and Recultivation, Konrad-Wachsmann-Allee 6, 03046 Cottbus, Germany  
 Stefan Lebzien, ENAGRA Biomasse GmbH, Auf der Grub 1, 54472 Monzelfeld, Germany