Interactive comments on "Changes of the CO_2 and CH_4 production potential of rewetted fens in the perspective of temporal vegetation shifts" by D. Zak et al.

We highly appreciate the work of both referees since it has raised some valid and interesting points that we were glad to re-assess. We were happy about the very positive comment of Referee 1 but also about the critical points of Referee 2 which has been helpful to overcome the shortcomings of the earlier text. The text has now been revised in accordance with the suggestions and comments as listed in detail below (referee comments in italics).

Anonymous Referee #1

Received and published: 3 November 2014

"This study brings valuable contribution to a better understanding of decomposition and nutrient fluxes processes after rewetting of fens. The results of this study can be applied in restoration planning and in post-restoration management in this type of ecosystem to influence final GHG emissions and leaching of nutrients. I especially appreciate very precise design of the whole experiment, when all potential effects of mesocosm incubation on final results were considered and discussed. The results are well discussed and compared with other studies. Implication for peatland restoration are also well described and discussed and conversion of results from mesocosm experiment to real scale is very important and useful. The conclusion are well justified by the data and nicely extend existing information"

We gratefully acknowledge this positive comment. We are very happy that the referee values the effort we have done with the experiment and to converse the results from the mesocosms to the real scale with great care. Indeed there have been some critical points detected by the Referee 2 which were very helpful to make some further improvements.

Anonymous Referee #2

Received and published: 14 January 2015

Comment R#2-1. *"This paper compares potential GHG emissions from litter from five macrophyte species. The experiment is fairly simple and mostly well described, and provides some useful in vitro data. However, because of the small scale and in vitro nature, there are limits to how the results can be extrapolated to the real world, and this need to be made clearer."*

We gratefully acknowledge this comment since it was our main concern during data evaluation to highlight the possible ecosystem implications as well as, to the same extend, the limitations of our results and to avoid the reader from over-interpreting our results. The referee noted the simplicity of our experiment, what we agree on. This simple approach allows (with limitations) the disentangling of the pure litter quality effect on methane emissions, but inherently bears the disadvantage that the tranfer of the results to a complex ecosystems is limited. In accordance with the referee this has to become clearer in the manuscript. Therefore we did a very critical re-assessment of the whole manuscript to ensure that the extrapolation of our experimental results is done in a reasonable way now (see also below). We liked the statement of the last part of the comment very much and used this phrases in a slightly modified form as a kind of a directive at the beginning of the second part of the discussion: ""Due to the small scale and in vitro nature of the incubation experiment, there are limits on extrapolating results to the "real world".

Comment R#2-2. *"Section 4.2 Implications for peatland restoration - Much of this section is speculation, and should be cut down considerably. It is completely unclear how the authors jump to estimations of annual net GHG exchange on an area basis. Literature values of annual biomass production are not very pertinent; it is the net balance of photosynthesis and ecosystem respiration that matters, plus the net methane emission. This should either be cut, or made explicitly clear how the estimations were done."*

This section was revised carefully. The parts related to GHG exchange were deleted. At the moment we solely focus on methane emission. Since we not measured all factors controlling in-situ methane emissions apart from the “substrate quality” we just summarized those factors instead of discussing them in detail. We still use the lab data to make an assessment of the annual methane production potential by using biomass data obtained from the sites under investigation. We believe that such an approach is reasonable to emphasize the importance of the decomposition of fresh shoot biomass from different wetland plants of the sites under investigation. In overall this part of the discussion is shortened by about half a page, the speculative statements were removed.

Comment R#2-3. *“Section 5 Conclusions - again, much of this is not deducible from the study described here. This should be restricted to what can be concluded from this study. Speculative extrapolation should be kept to the Discussion.”*

We agree some of the conclusions were not deducible from the study. Accordingly we revised the conclusions and believe that no speculations are left. However, we did not move those deleted aspects in the discussion in order to avoid including any further speculations to the manuscript.

Comment R#2-4. *„Statistical analysis - I don’t see the value of null-hypothesis testing here - the null hypothesis is not worth testing, and the sample size of n = 3 makes it somewhat futile. Showing confidence intervals on results would suffice. There is scope to look at statistical modelling of the GHG emissions in relation to litter composition and species, e.g. does including species in the model help explain variation in CH4 emission? This is far more relevant than presenting p-values of differences between species. Table 5 shows results of some regression analysis, but there is scope for more here, and this would improve the paper, ideally at the expense of some of the speculation in the Discussion.”*

The reviewer has likewise made a good point here. Therefore instead of using hypothesis testing, we have provided confidence intervals for the results in Tables 2, 3, and 4. We have removed the significance tests from the text. The confidence

intervals in Table 4 are based on the sample means of the daily measurements of gas emission rates to avoid pseudoreplication. We also agree with the reviewer that more statistical modelling of GHG emissions would be desirable, however this was not possible because (as described in the methods) the nutrient measurements were derived from pooled samples. Therefore it is not possible to do more than assess the correlation between species mean GHG emission rates and species mean nutrient content. This is what we show in Table 5 in connection with boxplots to illustrate species differences.

Comment R#2-5. *"There are a few more points that need clarification: GHG emissions are expressed as (for example) mg CO2-C per g C. However, how the denominator is calculated is ambiguous: is this based on the initial mass, the final mass or interpolated between these?"*

It is clarified (based on the initial mass) both in the text but also in the tables and figures.

Comment R#2-6. *"C. demersum seems to quite distinctly different from the other species stoichiometrically, with a very low carbon content. Firstly, it needs to be checked that such low values are actually plausible, and some reference given. Secondly, given the low C content, does it make sense to express results on a per g C basis? Do emissions from C. demersum appear high simply because of the low C content? Perhaps a total mass basis would be better."*

The carbon content of C. demersum was about 1.4 times lower than the other species, i.e. in average 343 mg/g dry mass which was in accordance with literature findings. We included this information in section 3.1: "The comparatively low carbon content of C. demersum, about 1.4 times less than the other plant species, is consistent with literature findings and holds also true for regions that presents tropical climate (e.g. Dos Santos Esteves and Suzuki, 2010)." We also related the emissions to dry mass and still found substantial differences.

Comment R#2-7. *"Figure 3 - what do the error bars represent?"*

Min and max values, which is denoted now.

Changes of the CO_2 and CH_4 production potential of rewetted fens in the perspective of temporal vegetation shifts

Dominik Zak 3/16/15 11:07 AM

Kommentar: Apart from the referee comments we checked the whole text again to delete any shortcomming regarding the writing but also shorten the introduction to some extent. In overall we believe that the text is muched improved over the last version. Again we gratefully acknowledged the comments of Referee 2!

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Abstract

Rewetting of long-term drained fens often results in the formation of eutrophic shallow lakes with an average water depth of less than 1 m. This is accompanied by a fast vegetation shift from cultivated grasses via submerged hydrophytes to helophytes. As a result of rapid plant dying and decomposition, these systems are highly-dynamic wetlands characterised by a high mobilisation of nutrients and elevated emissions of CO_2 and CH_4. However, the impact of specific plant species on these phenomena is not clear. Therefore we investigated the CO_2 and CH_4 production due to the subaqueous decomposition of shoot biomass of five selected plant species which represent different rewetting stages (*Phalaris arundinacea*, *Ceratophyllum demersum*, *Typha latifolia*, *Phragmites australis*, and *Carex riparia*) during a 154 day mesocosm study. Beside continuous gas flux measurements, we performed bulk chemical analysis of plant tissue, including carbon, nitrogen, phosphorus, and plant polymer dynamics. Plant specific mass losses after 154 days ranged from 25% (*P. australis*) to 64% (*C. demersum*). Substantial differences were found for the CH_4 production with highest values from decomposing *C. demersum* (0.4 g CH_4/kg dry mass day) that were about 70 times higher than CH_4 production from *C. riparia*. Thus, we found a strong divergence between mass loss of the litter and methane production during decomposition. If *C. demersum* as a

hydrophyte is included in the statistical analysis solely nutrient contents (nitrogen and phosphorus) explain varying GHG production of the different plant species while lignin and polyphenols demonstrate no significant impact at all. Taking data of annual biomass production as important carbon source for methanogens into account, high CH_4 emissions can be expected to last several decades as long as inundated and nutrient-rich conditions prevail. Different restoration measures like water level control, biomass extraction and top soil removal are discussed in the context of mitigation of CH_4 emissions from rewetted fens.

1 Introduction

Artificially drained minerotrophic peatlands, commonly called fens, are being rewetted on a large scale in many European countries, including Germany. For instance, an area in excess of 20,000 ha has been rewetted in the state of Mecklenburg-West Pomerania (north-east Germany) alone (Zerbe et al., 2013). The objectives behind rewetting include the reduction of greenhouse gas (GHG) emissions, in particular of carbon dioxide (CO_2) via oxidative degradation processes in the aerated peat soil, as well as the recovering of the nutrient sink and ecological habitat functions of pristine fens. As a result of long-term organic soil losses, subsidence and the associated lowering of the land surface, rewetting of these areas often results in shallow lake formation (Zak et al., 2010). These developing ecosystems differ considerably from pristine fens. Peat formation cannot occur in the open waterbody; instead the highly degraded submerged peat surface becomes covered by organic sediments which form readily due to the subaqueous decomposition of dying grassland vegetation that is intolerant to permanent flooding and the decomposition of shoot biomass from wetland plants (Hahn-Schöfl et al., 2011). With regard to lake ontogeny, these sites can be compared to lakes in the process of terrestrialization, where peat formation can follow as infill proceeds to surface levels (Benner and Escobar, 2009).

These newly formed shallow lakes with a highly degraded peat substrate are characteristically eutrophic and show high mobilisations of nutrients (phosphate and ammonium) and dissolved organic carbon (DOC) (Zak and Gelbrecht, 2007). Furthermore, extremely high methane (CH_4) emissions from rewetted fens have been observed (Hahn-Schöfl et al., 2011). It has been shown that severely degraded rewetted fens perform a net climate impact that exceeds that of drained fens (Chojnicki et al., 2007; Höper et al., 2008).

CH_4 as well as CO_2 formation results from anaerobic microbial decomposition processes and biogeochemical factors influencing the formation and release of these GHG gases from fens range from pH, nutrient status, temperature, the presence of alternative terminal electron

acceptors as well as, perhaps most importantly, the presence of microbially available reduced carbon (Bridgham et al., 2013). Most gaseous C production in pristine fens is derived from young plant litter and the litter quality, hereby defined as the microbial usability of the substrate, may differ substantially between plant species (Lai, 2009).

A systematic evaluation of the transferability of known links between site characteristics and gaseous C emissions from pristine fens to rewetted fens (i.e. shallow lakes over formerly drained peatlands) has not been accomplished so far. One distinct difference of rewetted fens from natural fens in Central Europe is the rapid secondary plant succession. In the initial phase of rewetting, *Phalaris arundinacea* has been observed to be the dominating plant species; more adapted to wet-dry conditions, this species routinely dies off within the first year of inundation (Hahn-Schöfl et al., 2011). Helophytes like *Typha latifolia* in marginal areas and *Ceratophyllum demersum* in the open waterbody have been observed to colonize the area within one or two years of rewetting (Steffenhagen et al., 2012). With increasing rewetting time, the peat forming plants *Phragmites australis* and various *Carex* species, such as *Carex riparia*, can become re-established (Zerbe et al., 2013). The influence of these predictable vegetation shifts on CO_2 and CH_4 emissions has not been studied yet.

In this study, the CO_2 and CH_4 production due to the subaqueous decomposition of these five most abundant plant species, which are considered to be representative of different rewetting stages, were investigated during a 154 day mesocosm study. Beside continuous gas flux measurements, bulk chemical analysis of plant tissue, including C, N, P, and plant polymer dynamics, were performed in order to gain further insights into changing litter characteristics. With respect to temporal vegetation shifts in rewetted fens, the results provide new insights into the mid-term climate effect of these ecosystems and will particularly be evaluated with regard to current management practices.

2 Materials and methods

2.1 Sampling sites

The sampling sites are located in the River Peene and River Spree valleys in north-east Germany (Fig. 1). Three formerly drained rewetted fens were chosen with rewetting times between 6 and 40 years. According to meteorological records from stations in Greifswald and Potsdam, the mean annual temperatures (January/July) were 1.2/18.1°C and 0.7/19.1°C and the mean annual precipitation was 596 and 582 mm along the River Peene and River Spree sampling sites between 1991 and 2007, respectively.

In the River Peene valley the rewetted fens "Zarnekow" and "Menzlin" were sampled. Under pristine conditions these fens were characterized as low nutrient percolation mires covered by brown moss-sedge-reed communities (Zerbe et al., 2013). In the 1960s a complex drainage system designed to intensify agricultural production lowered the water table to 2 m below the fen surface, thereby causing peat losses and changes in the physico-chemical peat characteristics (see Zak et al. (2008) for further details). Subsidence of the peat body by up to 1 m lowered the fen surface below the water levels of adjacent rivers. The initial fen vegetation was displaced by highly eutrophic grassland species like *P. arundinacea*. Rewetting of the former fen was initiated in 1995 under the auspices of the EU-funded conservation project "European Agriculture Guarantee Fund". The polder system was abandoned and dams were constructed in drainage ditches, resulting in large scale flooding of the area. Today the average water depth at the sampling sites ranges from 0.2 to 1.2 m, the vegetation includes *T. latifolia* and *C. demersum*.

The third sampling site is the former terrestrialization mire "Glieningmoor", located in the River Spree valley. Drainage activities started in the late 19th Century, however no polders or pumping stations were established. The resultant lowering of the water-table was of the order of several decimetres (< 1 m). Drainage and intensive agricultural use ceased in 1977 and the site was declared a nature protective area under a national designation. Non-inundated parts of the fen have already become re-colonized by a range of sedge species (*C. riparia, C. cespitosa, C. lasiocarpa, C. hartmanii, etc.*) as well as by few orchid species including *Dactylorhiza majalis* and *Epipactis palustris*, indicating a successful restoration towards to more natural conditions. Only a few, mostly marginal, areas chosen for plant sampling are influenced by infrequent inundation (water table up to 50 cm above soil surface).

Data concerning chemical characteristics of sites under investigation (soil properties and water quality) can be found in Table 1.

2.2 Plant sampling and sample preparation

Sampling of leaf and stem plant tissue was performed at the end of the vegetation period between October to November 2009. *T. latifolia*, *P. australis* and *P. arundinacea* (from marginal drier places) were collected from the Menzlin site. *C. demersum* was harvested from the Zarnekow site while *C. riparia* was obtained from Glieningmoor. All plant parts were transferred directly to the laboratory and air-dried at 30°C for 8 days. The dried materials were cut into pieces of 5-20 cm size and stored in shaded PVC-boxes at 20°C. An exception

to this treatment was applied to the submersed plant *C. demersum*. In contrast to emergent plants, the drying of the tissue of submerged aquatic plants before decomposition within the fen-waterbody is unlikely under natural conditions. To mimic the natural decomposition process in fens, the collected plant parts of *C. demersum* were stored in a PVC-box under site water until further utilization within a couple of days. Only a sub-sample of *C. demersum* was dried for chemical analysis.

Prior to the start of the incubation experiment, a leaching procedure was performed for all plant parts except *C. demersum*. This step was recognized to be necessary under the incubation conditions, as preliminary incubation experiments had shown substantial acidification of the water in the mesocosms, probably due to the decomposition of easily available organic compounds within the leachate. This step can further be rationalized as under natural conditions in rewetted fens senescent plant material will be subject to substantial leaching before reaching the anoxic detritus mud layer that was simulated in the mesososm experiment.

The following leaching procedure was performed: Four charges of 50.0 g air-dried plant material of each species (except *C. demersum*) were placed in 2 L polyethylene bottles. 1.5 L of 1.5 mM NaCl solution was added to each bottle resulting in complete inundation of the plant material. The bottles were closed and stored at room temperature with occasional manual agitation. The agitation was repeated at 4 h and again at 16 h by replacing the liquid phase with fresh 1.5 mM NaCl solution. In sum, three leaching steps were performed for each plant material. The wet plant tissues from 3 bottles of each tissue were placed directly into the mesocosms to initiate the decomposition experiment, while plant material from one treatment was dried until mass constancy at 90°C to determine mass losses via leaching (i.e. to determine the plant mass at the beginning of the incubation experiment) and to perform chemical analysis of the initial material.

2.3 Incubation experiment

The plant materials (including 50 g *C. demersum* without preceding leaching) were transferred into plastic cages (diameter 13 cm, length 20 cm, mesh size 1 cm) in order to fix the litter at the bottom of watertight PVC pipes (15 mesocosms in total; diameter 15 cm and length 35 cm; 3 replications per plant species). The *C. demersum* material was rinsed carefully with tap water before incubation to remove loose particles (silt and algae). To establish an active and similar microbial community in the mesocosms, 2 mL fresh detritus

mud taken solely from the sampling site Menzlin was added as slurry to the plant litter. Then 4.5 L of 3.4 mM $NaHCO_3$ solution was added to achieve a water level of about 10 cm below the top of the mesocosm. The buffer solution served to avoid osmotic stress for microorganisms and to prevent acidification of the solution during decomposition. The mesocsoms were closed with a gas-tight lid equipped with sampling ports for gas and surface water. The mesocosms were stored in a climate chamber at a constant temperature 15 ± 1°C in the shade for 154 days. This temperature was selected as it was shown to be more representative of the prevailing temperature of the surface sediment layer during the year rather than the average air temperature in these regions (~ 9°C). One explanation is that ground water discharge buffers in particular colder air temperatures in winter, but also much higher ones in summer. Oxygen concentrations, electrical conductivity (EC) and pH were measured in the water column directly above the incubated plant material using probes (WTW®). These measurements were performed after 3, 7, 21, 49, 104 and 154 days of incubation. Additional water samples (100 mL) were taken on day 7, 49 and 154 to analyse dissolved organic and inorganic carbon (DOC, DIC), dissolved nitrogen (DN), soluble reactive phosphorus (SRP), and total dissolved polyphenols. To avoid volume losses within the water body, the removed water was replaced by fresh solution of 3.4 mM $NaHCO_3$.

To measure CO_2 and CH_4 emissions a steady state flow through chamber system combined with automated gas analysis equipment was used as described in detail by Hahn-Schöfl et al. (2011). Briefly, a constant air flow of 6×10^{-3} m^3 h^{-1} was adjusted in the open headspace of the mesocosms. CO_2 and CH_4 emissions from the solutions with submerged litter were calculated by measuring ambient air concentrations and concentrations in the air flowing through the headspace of the mesocosms using an infrared multigasmonitor (Typ INNOVA 1312 from INNOVA AirTech Instruments, Ballerup, Denmark). To tackle the short term changes of gas release by ebullition three measurements per hour and per mesocosm were performed resulting in a total number of 72 samples per mesocosm and day. This enabled a more or less accurate tracking of total CO_2 and CH_4 emissions throughout the incubation period. According to our experimental design, we measured gross GHG emissions (i.e. production) due to litter decomposition; naturally occurring follow-up processes in fens, such as methane oxidation or detention within the mud, do not take place on a representative scale within this study. Therefore the transferability of our data to ‘real’ ecosystems is limited to the litter quality aspect and therefore the term GHG "production potential" is used in the following analysis and discussion. The calculated GHG flux is always based on the initial

mass or initial carbon content respectively. Mass related fluxes facilitate a better comparison with literature data.

Dominik Zak 3/16/15 11:18 AM
Kommentar: As requested by referee comment **R#2-5** we denoted that calculation is based on initial mass or initial carbon content respectively.

At the end of the incubation the remaining plant material was separated from the water by sieving (sieve mesh size: 1 mm). Afterwards, the plant material was freeze-dried and weighed. Ground samples of the plant material from each mesocosm were used for further chemical analysis.

In order to compare the different carbon fluxes during litter decomposition, the C-normalized carbon releases to water as DOC and DIC and to air as CO_2 and CH_4 were calculated on a percentual basis of total carbon loss from plant litter after 154 days. Carbon normalized accumulative amounts of CO_2 and CH_4 during the experiment were calculated. The DOC and DIC concentrations in the water at the end of the experiments were used to determine the amount of dissolved carbon released by the litter (water removals for analysis during the experiment were considered in the calculations).

2.4 Chemical analysis

Before chemical analysis of the different plant tissues the freeze-dried plant materials were homogenised with a cross hammer mill (Fritsch GmbH, Pulverisette 19 & 14, Idar-Oberstein, Germany). The total P content of ground plant materials were determined as SRP using the molybdenum blue method according to Murphy and Riley (1962) after an acid digestion procedure (10 mg dry sample + 2 ml 10 M H_2SO_4 + 4 ml 30 % H_2O_2 + 20 ml de-ionised water at 160°C for 2 h). Nitrogen and carbon contents of plant tissues were determined using an element analyzer (Vario EL by Elementar).

Total polyphenol contents of solids and water were determined colorimetrically using the Folin-Ciocalteau procedure slightly modified from Box (1983). In detail, for solid analysis approximately 200 mg of dried ground plant material was weighed in 40 mL centrifuge tubes. Extraction was performed by adding 10 mL of acetone (70%) for 20 min in an ultra sonic bath at 20°C. The extracts were then centrifuged at 10,600 *g* for 5 min. The extraction was repeated twice and 0.1 ml aliquots of the combined extracts were made up to volumes of 5 ml with distilled water in 10 ml screw cap glass tubes to obtain absorbance below 0.5. For the determination of total dissolved polyphenols in the liquid phase, water samples (0.2 - 2.5 mL) were taken from the mesocosms, 0.75 ml sodium carbonate solution (75 g Na_2CO_3 l^{-1}) and 0.25 ml Folin-Ciocalteau reagent (Merck KGaA, Darmstadt, Germany) were added and reaction mixtures were vortexed for 5 s. Absorbance was read at 750 nm (Photometer Spekol

221, Iskra Elektronik, Stuttgart, Germany) exactly 60 min after addition of the Folin-Ciocalteau reagent. The assay was calibrated with tannic acid (Fluka/Sigma-Aldrich, Munich, Germany). For determination of acid detergent cellulose and acid detergent lignin in following simplified denoted as 'cellulose' and 'lignin' the gravimetric method described in Gessner (2005) was applied.

The concentrations of DOC, DIC, and DN were analysed with a C/N-Analyser (TOC 5000, Shimadzu, Kyoto, Japan). The composition of the organic fractions in the water samples were determined for all plant species under investigation at days 7, 49 and 154 using liquid size-exclusion chromatography in combination with organic carbon detection (LC-OCD method, see Huber and Frimmel (1996)).

2.5 Statistical analysis

One way analysis of variance (ANOVA) was used with the plant species as factors to analyse CO_2 and CH_4 emissions, plant tissue characteristics (C, N, P, polyphenols, cellulose, and lignin) at the beginning and end of the experiments, as well as water chemistry (pH, EC, oxygen, SRP, TDP, DOC, DN, dissolved polyphenols) at days 7, 49 and 154 of incubation. CO_2 and CH_4 were expressed per unit carbon in plant biomass, although gas emissions based on plant dry mass were assessed. The results were not affected by the choice of biomass basis. Homogeneity of variance was checked using Levene's test. If variance differed significantly between species according to Levene's test, data were transformed using appropriate log or power transformation functions. Transformations were also performed occasionally even when Levene's test showed no significant difference in variance when the transformation visually produced substantially more homogeneous variance. If the ANOVA indicated a significant effect, Tukey's post-hoc test was used to analyse differences between individual species. To investigate whether plant tissue characteristics influenced GHG production, correlations with the mean values for each species were tested. Homogeneity of residual variance and influence of outliers using normalised residual plots and plots of Cook's distances were checked. All statistical analyses were performed with R version 3.0.1 (R Development Core Team, 2013).

3 Results

3.1 Plant litter quality and mass losses

The initial bulk parameters as well as bulk parameter changes during decomposition were variable among plant species whereby *C. demersum* differed substantially from the other species (Table 2). Litter dry mass loss after 154 days ranged from 25 to 64%. Litter from the submerged plant *C. demersum* showed the highest mass loss (64%) and a relative enrichment of the initially very low carbon content of 34% to 39% after 154 days. The comparatively low carbon content of *C. demersum*, about 1.4 times less than the other plant species, is consistent with literature findings and holds also true for regions that presents tropical climate (e.g. Dos Santos Esteves and Suzuki, 2010). Plant tissues from the other species had similar initial carbon contents ranging from 47 to 49%. These carbon contents remained fairly constant during decomposition, leading to C loss values similar to total mass loss (Table 2).

Fluctuations in the initial N content as well as changes during decomposition were much more distinct than C fluctuations. The initial N content of *C. demersum* at 2.8% was at least twice as high as the N contents of the other species and increased during decomposition to 4.3% by day 154. The other plant parts showed initial N content between 0.9% (*P. australis*) and 1.8% (*P. arundinacea*). At the end of the experiment, total N loss of the litter ranged from 40 to 60% for all plant species, with the exception of *P. australis* plant tissue, which showed a net increase of about 8% N, a finding that we cannot explain as no external N-sources were present (%N loss, Table 2). Net N losses exceeded net C losses for plant tissues that had small overall mass losses (i.e. *P. arundinacea*, *T. latifolia* and *C. riparia*), leading to increasing C/N-ratios. *C. demersum* in contrast lost 45% of its nitrogen, a value within the range of other plant species N-losses, but C-loss accounted for 59%, resulting in a C/N-decrease from 14 to 11.

At the start of the experiment, the P content of *C. demersum* was much higher than for the other plant species (0.68%) and *P. arundinacea* showed the lowest P content (0.04%). While species dependent differences in the initial P contents were observed, the P contents of all tissues had converged to the range of 0.04-0.05% by the end of the experiment with the exception of *C. demersum*, which stood out with an increase in P content to 0.96%.

The polyphenol content in *C. demersum* was higher than in tissues from other species by a factor of 2-3 at the start of experiments but lower at the end of experiments. *T. latifolia* and *P. australis* had the highest lignin and cellulose contents, followed by *C. riparia*,

Dominik Zak 3/16/15 9:57 AM

Kommentar: Comment R#2-4 to statistical analysis:

Instead of using hypothesis testing, we have provided confidence intervals for the results in Tables 2, 3, and 4. We have removed the significance tests from the text.

Dominik Zak 3/16/15 10:50 AM

Kommentar: According to the query of referee 2 (**R#2-6**) we checked the literature and found that our C content is reliable. The low value of C. demersum is also in accordance to values we measured previously at IGB. Later on depending on the question we show both data refer to mass conent or C content respectively. The differences we found are always high!

C. demersum, and *P. arundinacea.* After 154 days, however, the lignin content of *C. demersum* had increased to the highest value of all species.

The enrichment of lignin and the loss of polyphenols were common characteristics for all tissues, but again most pronounced for *C. demersum*.

3.2 Water quality

The water quality changed throughout the experiment with different patterns for each plant species and parameter (Table 3). Only the pH and oxygen values remained constant, with pH in the circum-neutral range (6.1 to 6.8) and oxygen around 1 mg L^{-1} for all plant species. One exception was *C. demersum*, where pH increased from 6.6 to 7.4 (Table 3). EC increased and concentrations of SRP and DN decreased during the course of experiments for all species (with the exception of *C. demersum*, where DN and SRP increased). EC and nutrients (SRP, DN) differed most for *C. demersum* during the experiments. EC was in the same range for all species on day 7 of incubation but was higher for *C. demersum* on day 154 by a factor of 7. Similarly, SRP and DN were similar for all species on day 7 but higher for *C. demersum* by a factor of 150 and 10, respectively. DOC concentrations on day 7 were higher for *C. riparia* and *P. arundinacea* than the other species but there were no differences in DOC at day 49 and 154. Polyphenol concentrations were lower on day 7 for *C. demersum* but there was no significant difference between species on day 49 and 154.

The DOC composition in the water for all plant species under investigation shows that low molecular weight substances constituted the highest proportion throughout the incubation period (Fig. 2).

3.3 Production of CO_2 and CH_4

The daily average GHG production due to the 154 day decomposition of the different plant species ranged from 0.29 to 0.68 mg C/g dry mass for CO_2 and from 0.004 to 0.3 mg C/g dry mass for CH_4 (Table 4, Fig. 3). The cumulative gas production (CO_2, CH_4, and CO_2+CH_4) in relation to plant carbon content was highest for *C. demersum* (Fig. 4). *P. arundinacea* showed the second highest CO_2 production followed by *C. riparia, P. australis* and *T. latifolia* and a comparably high CH_4 production. *P. australis* had higher CH_4 and total GHG production than *C. riparia, T. latifolia* and *P. arundinacea* ($p < 0.01$).

Statistical analysis suggested that the gaseous C production (CO_2, CH_4, and CO_2+CH_4) depends on the nutrient (N, P) content of plants. The gaseous C production correlated

positively with N and P, regardless of whether N or P content was considered at the start or at the end of the experiment, or whether CO_2, CH_4 or the sum of both was considered ($p < 0.01$). This result also did not depend on whether the mass basis for the calculation was dry mass or carbon content (Table 5). It should be noted here that the statistical significance is due to a large part to *C. demersum*, which constituted an outlier due to its high nutrient release and gas emission. Repeating the analysis with the non-parametric Kendall rank correlation test yielded no significant relationships (Table 5). Polyphenols, lignin, and cellulose had no statistical correlation to gaseous C production.

For all plant species CO_2 production was the major pathway for total C losses, accounting for about 30 to 44% while the production of CH_4 made up only a minor portion of C losses (Table 4). Only in the case of *C. demersum* CH_4 did substantially contribute to C loss (20%). The DOC and DIC production (determined at the end of the incubation) accounted for 4.1 to 7.7% and 0.6 to 14.3% of the total C losses, respectively. A substantial part of carbon losses, for some plant species a major part, could not be quantified by gaseous C production and DOC/DIC release (Table 4). This 'balance gap' might in part be attributed to the release of fine particulate organic matter smaller than 1 mm (mesh size of sieve for removal of plant residuals, see section 2.4) and to the release of volatile organic compounds (e.g. Bäckstrand et al., 2010).

4 Discussion

The wetland plant species considered in this study (*P. arundinacea, C. demersum, T. latifolia*, *P. australis,* and *C. riparia*) have fast metabolism and growth and become abundant at different rewetting stages in inundated peatlands (Zerbe et al., 2013). In vigorous stands, the annual shoot biomass production may exceed 0.1-2 kg of dry mass per square meter, consequently altering the carbon and nutrient cycles of these newly formed ecosystems (Steffenhagen et al., 2012; Zak et al., 2014). The senescent shoot biomass of these plant species (harvested at the end of the vegetation period) were chosen for an incubation experiment to elucidate their importance for elevated GHG emissions from inundated peatlands (Koch et al., 2014) depending on the chemical composition of plant litter.

4.1 Litter breakdown and greenhouse gas production

Litter breakdown and GHG production of shoot biomass under natural conditions is the result of a distinct sequence of differing processes which were widely mimicked in this study. Firstly, the "leaching stage" which occurs subsequent to die back of plants causing high mass

losses of plant nutrient stock (Gessner, 1991; Aerts and De Caluwe, 1997), hydrolysable polyphenols and other mostly low molecular weight organic substances such as carbohydrates and amino acids (Best et al., 1990; Maie et al., 2006); secondly, the comparatively fast decomposition under aerobic conditions before and after collapse of shoot biomass into the surface water; and, finally, the slowed decomposition of plant litter under prevailing low-oxygen or even anaerobic conditions if submerged plant litter reaches the newly formed detritus mud layer (Asaeda et al., 2002). It is not possible to distinguish clearly if, or when, the subsequent decomposition of plant litter takes place mainly under anaerobic conditions in the experiment described. However, low-oxygen concentrations in the surface water layer (Table 3) and the release of CH_4 during the entire incubation period with a lag phase of about four weeks at the onset of the incubation (Fig. 4) indicates that at least part of the incubated litter was decayed anaerobically. In particular for *C. demersum* it can be assumed that anaerobic conditions dominated due to the narrow production ratio of CO_2 versus CH_4 of about two (see below). It is also important to consider that in-situ litter breakdown is driven by various detritivorous macroinvertebrates called shredders (Hieber and Gessner, 2002), however these were not present in the experiment. Therefore, and due to other reasons, it is necessary to be cautious if transferring the experimental results to natural field conditions (see section 4.2).

In accordance with previous studies, it was found that leaching contributed to major P losses for all tested plant species (in average 50 to 80% of initial P mass), but also for N with losses up to about 40%, as recorded for *P. australis,* and to some extent similarly for C but at much lower rates (single results are not shown). There is some evidence that part of the high leaching losses were supported by the prior drying of the plant litter (Gessner, 1991), however drying is a naturally occurring process in these systems where emergent helophytes are yet to collapse and become submerged. In addition, substantial parts of potentially enzyme inhibiting polyphenols become released so that the overall leaching may strongly impair the decomposability of plant litter in one direction or the other. Therefore the litter quality of the leached material was chosen (with exception of *C. demersum*) to test the importance of different plant compounds on the detected mass losses and GHG production. While there is clear evidence that prior drying affects the decomposition of plant litter due to substantial leaching we assume that possible differences compared to fresh incubated plant litter (herein *C. demersum*) become balanced in the longer term i.e. in order of weeks and months as tested in this study.

Different models exist that aim to predict GHG emissions from inundated wetlands. However, some of the models simply consider the water table as a key factor (Couwenberg et al., 2011), while more local adapted models include environmental parameters including vegetation, net primary production and average mass losses due to decomposition (Potter et al., 2011). Despite its importance, the influence of plant specific litter quality on GHG production in wetlands remains poorly understood and litter quality is thus treated as a constant in most GHG emission models (Bridgham et al., 2013). In the present study, the plant specific mass losses due to subaqueous litter decomposition varied by a factor of three while for instance the CH_4 emissions varied by a factor of 70. *P. australis* showed the lowest mass loss of all tissues, but the second highest methane production. This indicates, that the litter quality of the plant parts were highly distinct, but more importantly that there is no simple linear relationship between mass loss and CH_4 production concerning litter from different plant species. Low litter quality, if defined by low mass loss during decomposition, does not imply low methane production.

There exists the possibility of a traditional explanation of the above mentioned divergence of mass loss and CH_4 emissions between plant species. The experimental design of this subaqueous decomposition study was not completely anaerobic, as the water was not initially deoxygenated and diffusion of oxygen from the air into the water was possible all times, as it might occur under natural conditions. Methanogenesis is suppressed by more thermodynamically favourable metabolic pathways in the presence of alternative terminal electron acceptors (TEA), especially oxygen (Bridgham et al., 2013). Assuming a certain amount of oxygen initially present and a constant diffusion of oxygen from the air into the waterbody, CH_4 production due to decomposition would be preferentially suppressed in plant tissues with a low carbon quality that decompose at a slower rate. As a result, the CO_2 : CH_4 ratios would be higher for more slowly decomposing plant parts and the CH_4 : mass loss ratio would be lower. In an exclusively fermentative and methanogenic system the CO_2 : CH_4 ratio should be ca. 1 : 1 (Keller et al., 2009). In the present study, *C. demersum* had the highest mass loss and showed a CH_4 : CO_2 ratio of 2.3, however, the second highest CH_4 : CO_2 ratio was found for *P. australis*, the tissue with the lowest mass loss of all species (i.e. lowest quality) in this experiment (Table 4). Consequently, the differences in CH_4 emissions in relation to mass loss in this study were most likely not primarily a result of different TEA supply but a function of litter quality.

The concepts of litter quality and their overall importance in the decomposition process of fresh litter have been widely studied in lakes, wetlands and other aquatic environments

(Gessner, 2000; Hieber and Gessner, 2002). Plant litter high in cellulose, hemicellulose, and sugars and low lignin content decompose at a faster rate than litter with high lignin content (Reddy and DeLaune, 2008). In this study, the enrichment of lignin concurred with the mass loss (Table 2), indicating the recalcitrance of this biopolymer. Cellulose content decreases in *C. demersum*, even if the overall carbon content of this tissue increases, but increases for *P. australis* were detected and for other plants the content remains fairly constant. Thus the data show common relationships between biopolymer dynamics and mass loss during decomposition, but no correlations with the observed CH_4 emissions. Polyphenols are another class of substances that can inhibit decomposition processes (Freeman et al., 2001) but our data showed no correlation between mass loss or GHG emissions and polyphenol content.

N dynamics are an additional important aspect of litter decomposition. Decomposing microorganisms depend on N sources for their anabolism and increasing concentrations of N within the litter suggest microbial activity (Tremblay and Benner, 2006). Actually, the sole N source in this study was the plant tissue itself, thus no external nitrogen fixation could occur. However, atmospheric N could explain the increased amount of N for *P. australis*, however if this source can be relevant needs further investigations. All plant tissues except *P. australis* lost between 40 and 60% of their N content which were in part recovered within the water phase as dissolved N (Table 2 & 3). This indicates that the decomposition process of all tissues was not N limited. A decrease of the C/N ratio was observed for *C. demersum* and *P. australis*, but due to the data basis we could not assign if this relative enrichment of N was due to the high C loss or to microbial N fixation and if any link to the high CH_4 production was present.

It has been suggested that CO_2 and CH_4 production due to anaerobic respiration in fens is primary from dissolved organic matter and fresh carbon inputs (Bridgham et al., 2013). In line with this assumption we found that the dominant fraction of DOC in this study consisted of low molecular substances like acetate that can directly serve as substrates for methanogens (Fig. 2). The present distribution of DOC molecular weight is very different from the distribution found in natural environments that consists primarily of high molecular weight substances (Zak et al., 2004). We therefore suggest that this fresh DOC is highly labile and indeed plays a dominant role in the measured GHG production. Consequently the concentrations of DOC as measured three times during the decomposition experiment are not representative for the DOC released from the litter but are the sum of DOC release and DOC respiration (Table 3). This would explain why the DOC concentrations remained fairly small and constant for all species over time despite the great differences in litter mass loss. Most

notable is the enrichment of DIC during the decomposition of *C. demersum* that showed the highest mass loss and the highest GHG production from all species but showed no enrichment of DOC.

4.2 Implications for peatland restoration

Due to the small scale and in vitro nature of the incubation experiment, there are limits on extrapolating results to the "real world". The following section considers other variables controlling carbon fluxes under in-situ conditions and discusses different restoration measures to mitigate GHG emission, in particular of CH_4, based on our findings.

As documented from other similar wetland types, such as shallow lakes, it was assumed that in the aquatic systems investigated the die-off of submerged and emergent macrophytes at the end of the growing season is the primary source of detritus production and therefore contributes significantly to biogenic gas production, while the release of methane by degraded peat at the fen surface and deeper less decomposed peat can be neglected (Hahn-Schöfl et al., 2011). It addition, it was shown that the net CO_2 exchange becomes negative shortly after rewetting of degraded peatlands, but the lake formation generates CH_4 hot spots (Wilson et al., 2009). Although the re-establishment of the C sink function can be rapid and substantial in inundated peatlands (Cabezas et al., 2014), the release of CH_4, with a 25 times higher global warming potential, results in a "net warming effect". Therefore further consideration of CH_4 is required.

Among the plant species investigated, *C. demersum* had the highest CH_4 production potential, but the recorded biomass production was 6 to 16 times lower on average than the helophytes under investigation (Steffenhagen et al., 2012). Less aboveground biomass leads to lower detritus production and hence to less substrate for methanogenesis. Accordingly, taking data of the annual biomass production of the sampled 'Peene sites' and the CH_4 production potential (determined at 15 °C) together, the annual CH_4 production potential would account for about 150 kg CH_4/ha for *C. demersum* and about 250 kg CH_4/ha for *P. australis* (Table 6). However, it should be noted that biomass production of *C. demersum* can be much higher depending on specific site conditions (Küchler, 1986). The estimated annual CH_4 release for *P. australis* is in the middle of the range of emissions determined for a *P. australis* stand recorded recently in-situ for an inundated coastal brackish fen (46 – 1,329 kg CH_4/ha a) located in the neighbourhood of the investigated 'Peene sites' (Koch et al., 2014). A maximum rate of about five times higher cannot be explained by differences in biomass

Dominik Zak 3/16/15 9:45 AM

Kommentar: According to the second referee comment R#2-2 we revised this part of the discussion carefully. Changes include:

1)Parts related to GHG exchange were deleted.
2) Instead of discussing all factos controlling in-situ methane emissions we summarized those factors at the end of this section
3) Speculative statements were removed

Dominik Zak 3/16/15 10:55 AM

Kommentar: We deleted following parts: The experiments found that the production of CO_2 and CH_4 by litter decay varied with plant species in the following descending order *C. demersum* >> *P. australis* > *T. latifolia* > *P. arundinacea* > *C. riparia* (Table 4). The order changes if only mass losses are considered but the highest values were still recorded for *C. demersum* and the lowest for *P. australis*. In face of this finding, restoration measures which seek to mitigate GHG production should be oriented towards fast re-colonization with *C. riparia* while avoiding high abundances of *C. demersum*. However, in order to discuss different restoration measures to mitigate GHG emissions resulting from these experimental results, it is necessary to consider other variables controlling carbon fluxes under in-situ conditions. The gaseous C fluxes or GHG budget respectively are a function of primary production, i.e. net uptake of CO_2 mainly during the growing season from May to September and autotrophic as well as heterotrophic respiration processes producing CO_2 and CH_4 throughout the year (Bäckstrand et al., 2010). For the total carbon balance, the net fluxes of dissolved organic and inorganic carbon must be considered and these depend strongly on the prevailing hydrological conditions as do the gaseous C fluxes.

production but may imply that other sources or variables contribute to high CH_4 emissions. Apart from the decomposition of fresh detritus organic matter, the older accumulated detritus material might contribute to CH_4 production in the course of sediment diagenetic processes as well as the consumption of dead root material and organic compounds leached from roots (Potter et al., 2014). Other variables which influence the in-situ methane emissions include: i) oxygen release in the rhizosphere of emergent helophytes so that a major part of the produced CH_4 may become oxidised, ii) the methane transport through plant aerenchyma, iii) seasonal and spatial changes of temperature as well as methanogen or methanotroph community and, iv) the water quality, i.e. the level of sulfate concentrations (Fritz et al., 2011; Bridgham et al., 2013; Koch et al., 2014).

Despite of the complexity of factors controlling in-situ methane emissions experimental findings enable us to discuss different restoration measures, including water level control, biomass extraction and top soil removal.

1. Water level was found to be a main driver for CH_4 emissions from peat soils (Kim et al., 2012). In terms of peatland restoration, the optimum would be to adjust water tables to the surface or just below, thus preventing inundated conditions as far as possible (Joosten et al., 2012). Such an approach would facilitate conditions for new peat formation and elevated CH_4 emissions would be unlikely as shoot biomass would be decomposed primarily under aerobic conditions. However, the subsidence and peat loss by several decimetres, the damage of the oscillation capability, and a pronounced microtopography of long-term drained areas results in the formation of shallow lakes with water depths which vary spatially from a few centimetres to several decimetres, even across short distances, render a single water depth unfeasible. If the water table can be managed, e.g. by installing a dam or controlling the water outlet from the peatland, the water table depth should not exceed 0.5 m since this is the minimum depth required for *C. demersum* to grow (Hutchinson, 1975).

2) In most cases, water level management is both economically and technically unfeasible so that the harvesting and removal of aboveground biomass should be considered as an option to mitigate CH_4 emissions. The removal of *P. arundinacea* and other grassland species including sod cutting before rewetting would strongly reduce the initial high CH_4 emissions within the first one or two years of inundation (Hahn-Schöfl et al., 2011). Harvesting of submerged and emerged wetland plants might be also be feasible, however this may be technically more challenging and would potentially carry a high cost. Therefore the use of plant material for biogas or in the case of *P. australis* additionally as fodder, building material or other purposes

Dominik Zak 3/16/15 11:01 AM

Kommentar: The previous part was:

Apart from the decomposition of fresh detritus organic matter, the older accumulated detritus material might contribute to CH_4 production in the course of sediment diagenetic processes as well as the consumption of dead root material and organic compounds leached from roots (Potter et al., 2014). Whereas the role of highly degraded and underlying, less decomposed peat can be widely neglected (Hahn-Schöfl et al., 2011). On the other hand, all of the investigated emergent helophytes are known to release oxygen in their rhizosphere so that a major part of the produced CH_4 may become oxidised by methanotrophic bacteria (Fritz et al., 2011). But, at the other hand a major part of produced methane may be bypassed by transport through plant aerenchyma (Bridgham et al., 2013). All of these phenomena can be excluded for the cultivated grassland species *P. arundinacea* and for the submerged hydrophyte *C. demersum.* Accordingly, we assume that CH_4 production potential can be roughly equated to CH_4 emissions for these plants.

(Joosten et al., 2012) can be useful to offset these costs. However, the removal of above ground plant material would also reduce siltation rates within the shallow lakes so that the re-colonisation with peat-forming plants adapted to widely non-inundated conditions, such as low sedges and brown mosses, can be retarded.

3) The removal of upper degraded peat soils often only less than 30 cm thick before rewetting would minimise the nutrient availability for plants and would also provide a re-colonisation of plants adapted to more nutrient poor conditions (Emsens et al., 2015). This so-called top soil removal could potentially lower substantially the biomass production and additionally the chemical composition of plant material might change towards a more refractory character, however this needs further investigation.

5 Conclusions

Dominik Zak 3/16/15 11:03 AM

Kommentar: According to the third referee comment R#2-3 we removed the thougths which were not deducible from the study:
We removed:
"This study suggests that submerged hydrophytes generate higher CH_4 emissions than helophytes." We believe that all other parts can be concluded from the study.

The typical temporal vegetation shifts in inundated formerly drained fens from cultivated grasses via submerged hydrophytes to helophytes will strongly alter the GHG emission potential. This study shows that *C. demersum* a dominating hydrophyte in open shallow waters of the initial stage of rewetted fens generates higher CH_4 emissions than helophytes under investigation. However, even as succession towards plants with a lower GHG production potential like *P. australis* occurs, high CH_4 emissions in rewetted fens can be expected to continue for several decades as long as inundated conditions prevail. It is important to note that high mass losses in the course of litter decay cannot be equated with a high CH_4 emission. Further investigations are needed to show how other common wetland vegetation, for example floating macrophytes like Lemnacea or other *Carex* species adapted to inundated conditions, contribute to GHG emissions. In addition, it is not clear yet to which extent a lowering of nutrient conditions would affect the decomposability of plant litter in inundated fens. To answer this question, it is recommendable to obtain plant samples from inundated sites where degraded nutrient enriched top soil has been removed.

Author contribution

D. Z., J. G., M. B. and J. A. designed the experiment; M. B. and D. Z. carried out the experiments; T. S. and H. R. did data analysis; D. Z. and H. R. prepared the manuscript with contributions from all co-authors.

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Table 1. Selected data on chemical composition of surface water (SW), soil pore water (PW), and peat of the sites under investigation (EC = electrical conductivity, SRP = soluble reactive phosphorus, N = nitrogen, Ca = calcium, and C = carbon). A detailed description of peat and water sampling can be found in Zak et al. (2010). Peat data from two different soil depths are given as medians of always three sub samples.

	Sites/year of rewetting	'Zarnekow'/2004		'Menzlin'/2002		'Glieningmoor'/1977	
SW	Sampling time	March 2004 – July 2012		April 2003 – July 2012		July 2008 –May 2013	
	Number of samples	36		12		40	
	Water depth	ca. 0.2 – 1.2 m		ca. 0.3 – 0.5 m		ca. 0.0 – 0.6 m	
		Median	Range	Median	Range	Median	Range
	pH	7.7	6.4-9.0	7.4	6.8-9.1	7.2	6.7-8.5
	EC (µS/cm)	834	522-1127	1201	684-1513	468	267-674
	SRP (mg/L)	0.44	0.02-2.88	0.98	0.17-2.19	0.01	0.003-0.039
	Nitrate-N (mg/L)	0.05	0.01-5.2	0.05	0.03-0.06	0.01	0.01-0.13
PW	Sampling time	March 2004 – July 2012		April 2003 – July 2012		December 2009	
	Sampling depth	0-60 cm		0-60 cm		0-60 cm	
	Number of samples	201		39		3	
		Median	Range	Median	Range	Median	Range
	pH	6.9	6.3-7.4	6.6	6.3-6.9	6.2	6.1-6.3
	EC (µS/cm)	2320	1083-4850	1046	690-1503	309	305-368
	SRP (mg/L)	1.01	0.49-6.18	8.80	1.95-18.90	0.23	0.15-0.26
	NH_4^+-N (mg/L)	11.1	6.0-19.3	9.4	2.2-22.5	1.1	0.8-1.2
	Ca (mg/L)	709	293-1185	145	88-218	39	37-47
Peat	Sampling time	August 2004		February 2005		December 2009	
	Number of samples	3	3	3	3	3	3
	sampling depth	0-30 cm	30-60 cm	0-20 cm	20-60 cm	0-20 cm	20-60 cm
	Peat degradation	amorph, earthified	moderately decomposed	amorph, earthified	moderately decomposed	amorph, earthified	highly decomposed
	Parent material	unidenti-fiable	tall sedges	unidenti-fiable	reed, tall sedges	unidenti-fiable	sedges, mosses
	C content (mg/g DM)	398	444	381	510	186	380

N content (mg/g DM)	34	28	32	32	14	25
P content (mg/g DM)	1.34	0.54	2.01	0.55	0.94	0.63

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Table 2. Plant litter parameters at the start and at the end (= 154d) of the experiment and mass losses of total mass (TM), carbon (C), nitrogen (N), phosphorus (P) throughout the incubation. All values are related to dry mass and given as means (n = 3). ADC = acid detergent cellulose, ADL = acid detergent lignin. 95% confidence intervals are given in parentheses (for some parameter calculation was not feasible).

Dominik Zak 3/16/15 10:02 AM
Kommentar: As suggested by referee comment **R#2-4** we have provided confidence intervals now.

Parameter	Time of sampling	*P. arundinacea*	*C. demersum*	*T. latifolia*	*P. australis*	*C. riparia*
% TM loss	end	*44.6*	*64.0*	*36.8*	*25.1*	*30.8*
% C loss	end	*44.8*	*58.8*	*36.0*	*26.3*	*29.2*
% N loss	end	*63.1*	*44.7*	*47.8*	*-8.5*	*39.5*
% P loss	end	*44.6*	*49.6*	*63.5*	*50.1*	*42.3*
C%	start	47.2 (45.9-48.5)	34.3 (26.4-42.2)	47.5 (46.2-48.8)	48.0 (47.3-48.6)	48.4 (48.1-48.7)
	end	47.1 (46.4-47.7)	39.3 (37.3-41.3)	48.1 (47.9-48.3)	47.2 (47.0-47.4)	49.5 (49.3-49.7)
N%	start	1.8 (1.5-2.1)	2.8 (2.6-2.9)	1.1 (1.1-1.2)	0.9 (0.9-1.0)	1.6 (1.3-1.9)
	end	1.2 (1.0-1.5)	4.3 (4.1-4.6)	0.9 (0.6-1.3)	1.1 (1.07-1.14)	1.4 (0.9-1.9)
P%	start	0.04 (0.04-0.05)	0.68 (0.36-0.99)	0.07 (0.04-0.09)	0.06 (0.05-0.08)	0.06 (0.05-0.07)
	end	0.04 (0.03-0.06)	0.96 (0.54-1.37)	0.04 (0.04-0.05)	0.04 (0.03-0.04)	0.05 (0.03-0.07)
%ADL	start	2.4 (2.2-2.6)	3.0 (1.7-4.3))	7.7 (7.5-8.0)	11.1 (10.2-12.1)	5.0 (4.3-5.7)
	end	5.7 (4.9-6.5)	19.2 (15.3-23.1)	11.9 (10.8-13.0)	16.0 (15.6-16.4)	8.3 (7.8-8.8)
%ADC	start	39.4 (38.8-39.9)	24.7 (22.7-26.7)	47.1 (46.8-47.5)	47.6 (46.3-48.9)	45.2 (43.6-46.8)
	final	38.1 (35.5-40.7)	20.8 (18.9-22.6)	43.7 (42.0-45.4)	58.0 (57.3-58.7)	42.6 (38.1-47.1)
%Polyphenols	start	1.5 (1.4-1.6)	5.8 (4.8-6.8)	4.1 (3.9-4.3)	1.5 (1.3-1.6)	2.9 (2.6-3.2)
	end	0.1 (0.08-0.11)	0.07 (0.03-0.10)	2.0 (1.5-2.6)	1.2 (1.1-1.3)	2.5 (2.3-2.8)
C/N (mole)	start	31	14	49	60	35
	end	46	11	61	50	43
ADL/N	start	1.3	1.1	7.0	12.3	3.1
	end	4.8	4.5	13.2	14.5	5.9

Table 3. Chemical composition of the water phase of incubated submerged plant litter at different sampling days (n.d. = not detectable, n.a. = not analysed). Values are given as means (n = 3), 95% confidence intervals are given in parentheses (for some parameter calculation was not feasible, negative values are set to zero).

Dominik Zak 3/16/15 10:03 AM
Kommentar: As suggested by referee comment **R#2-4** we have provided confidence intervals now.

Parameter*	Sampling day	*P. arundinacea*	*C. demersum*	*T. latifolia*	*P. australis*	*C. riparia*
pH	7	6.4 (6.2-6.7)	6.6 (5.9-7.3)	6.8 (6.3-7.4)	6.6 (6.4-6.7)	6.5 (6.3-6.6)
	49	6.1 (5.7-6.5)	6.7 (6.0-7.4)	6.7 (6.6-6.9)	6.5 (6.2-6.8)	6.3 (5.4-7.2)
	154	6.3 (5.9-6.7)	7.4 (7.1-7.7)	6.7 (6.0-7.3)	6.8 (6.3-7.4)	6.5 (5.7-7.2)
EC (µS/cm)	7	366 (345-386)	553 (423-683)	452 (400-504)	351 (340-362)	410 (399-421)
	49	396 (361-431)	1741 (1610-1880)	546 (526-566)	347 (340-355)	494 (477-512)
	154	425 (297-553)	3510 (3350-3670)	618 (587-649)	373 (357-388)	474 (441-507)
O_2 (mg/L)	7	1.0 (0.9-1.2)	n.d.	1.3 (0.2-2.5)	1.3 (0.5-2.1)	1.1 (0.1-2.0)
	49	1.3 (1.1-1.4)	n.d.	1.4 (1.4-1.4)	0.9 (0.0-1.8)	1.4 (0.4-2.3)
	154	1.3 (0.8-1.8)	n.d.	1.1 (0.4-1.8)	1.1 (0.5-1.6)	1.0 (0.3-1.7)
SRP (mg/L)	7	0.87 (0.5-1.3)	1.53 (0.0-3.1)	1.13 (0.5-1.8)	0.28 (0.1-0.5)	1.32 (0.7-2.0)
	49	0.14 (0.0-0.4)	17.30 (0-39)	0.96 (0-1.9)	0.04 (0.03-0.04)	0.35 (0-1.13)
	154	0.07 (-0.01-0.15)	16.98 (4.2-29.7)	0.17 (0-0.33)	0.08 (0.03-0.13)	0.15 (0-0.44)
DOC (mg/L)	7	106 (74-138)	32 (13-51)	53 (32-74)	45 (32-57)	95 (69-120)
	49	129 (60-199)	93 (-28-214)	49 (41-57)	94 (65-123)	111 (0-233)
	154	135 (-8-277)	105 (52-158)	90 (10-170)	103 (81-126)	93 (0-202)
DIC (mg/L)	7	44	62	51	45	42
	49	52	n.a.	69	41	68
	154	55	418	73	49	58
DN (mg/L)	7	4.1 (2.9-5.4)	2.3 (1.6-3.1)	3.0 (1-5)	3.3 (1-6)	3.7 (2.0-5.4)
	49	7.6 (3.5-11.6)	25.8 (17-35)	1.2 (0.6-1.8)	5.4 (1-10)	9.8 (7.3-12.2)
	154	14.0 (0-31)	112 (89-135)	3.8 (-2-10)	14.4 (8-21)	9.3 (0-18)
Polyphenols	7	20.1 (15-25)	3.1 (0-6)	22.5 (17-28)	14.9 (9-21)	23.9 (12-36)
(mg/L)	49	26.1 (7-46)	n.a.	10.7 (6-16)	n.a.	14.1 (5-23)
	154	24.6 (0-50)	n.a.	18.6 (0-40)	n.a.	13.9 (8-20)

* EC = electrical conductivity; SRP = soluble reactive phosphorus; DOC = dissolved organic carbon; DIC = dissolved inorganic carbon; DN = dissolved nitrogen.

Table 4. Daily averages of Gaseous carbon (C) production due to the 154 day decomposition of different wetland plant (given as mg C/g dry mass for CO_2 and as µg C/g dry mass for CH_4) and carbon balance for the decomposition experiment as calculated by carbon losses from tissues via gaseous and aqueous pathways (calculation is based on initial dry mass). Values of carbon losses and balance gap are given in percent of total carbon loss from the plant tissue due to decomposition. Values in parentheses are 95% confidence intervals.

Dominik Zak 3/16/15 10:12 AM
Kommentar: As requested by referee comment **R#2-5** we denoted that calculation is based on initial mass.

Dominik Zak 3/16/15 10:03 AM
Kommentar: As suggested by referee comment **R#2-4** we have provided confidence intervals now.

Parameter	*P. arundinacea*	*C. demersum*	*T. latifolia*	*P. australis*	*C. riparia*
CO_2 production	0.37 (0.33-0.41)	0.68 (0.44-0.93)	0.31 (0.21-0.41)	0.34 (0.28-0.40)	0.29 (0.20-0.38)
CH_4 production	9.4 (4.1-14.7)	302.7 (249-356)	8.1 (3.1-13.0)	30.8 (23.1-38.6)	4.3 (-2.3-11.0)
CO_2 : CH_4 ratio	40	2.3	38	11	67
C loss via CO_2 / %	29.7	43.9	30.3	41.8	32.9
C loss via CH_4 / %	0.7	19.5	0.8	3.8	0.5
C loss via DOC / %	7.7	4.1	5.3	7.7	6.8
C loss via DIC / %	0.6	14.3	1.9	0.6	1.2
balance gap / %	62.3	18.2	61.7	46.1	58.5

DOC = dissolved organic carbon; DIC = dissolved inorganic carbon.

Table 5. Significance level (p values) of correlations between contents of nitrogen (N) or phosphorus (P) and greenhouse gas (GHG) production considering litter characteristics at the beginning of the experiment ('start') and at the end of the experiment ('final'). The mean GHG production rates of each species (*Phalaris arundinacea*, *Ceratophyllum demersum*, *Typha latifolia*, *Phragmites australis*, and *Carex riparia*) were related either to dry mass or to C content. Due to the high influence of *C. demersum*, we tested correlations with both the Pearson correlation test (PCT) and the non-parametric Mann-Kendall test (MKT, *C. demersum* was excluded). Polyphenols, lignin and cellulose had no significant effect on GHG production.

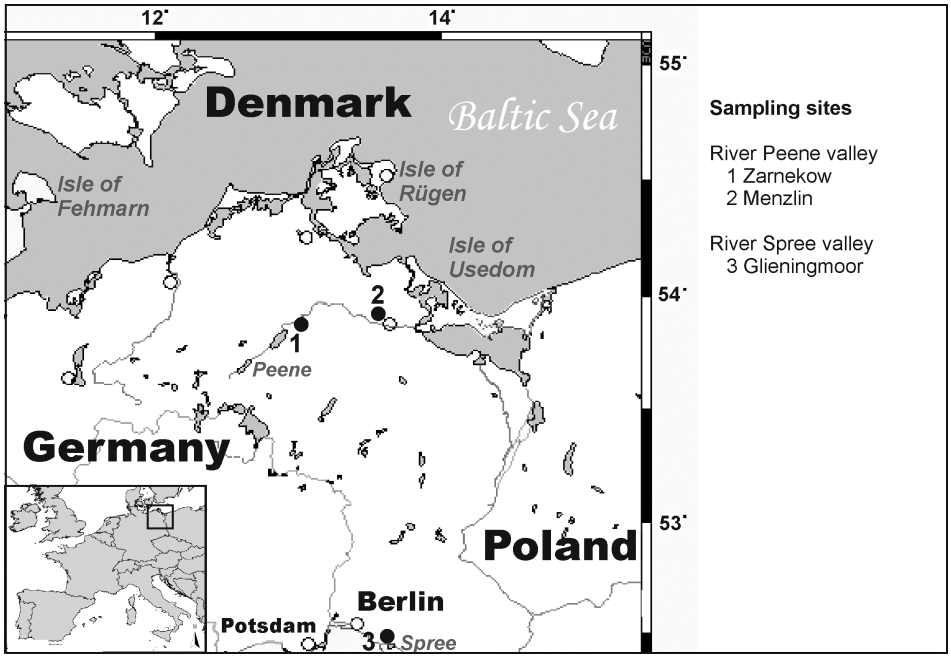
Dominik Zak 3/16/15 10:06 AM
Kommentar: R#2-4 asked for doing mor out of these results; however this was not possible because (as described in the methods) the nutrient measurements were derived from pooled samples. Therefore it is not possible to do more than assess the correlation between species mean GHG emission rates and species mean nutrient content.

	start						final					
	CO_2		CH_4		CO_2+CH_4		CO_2		CH_4		CO_2+CH_4	
	PCT	MKT	PCT	MKT	PCT	MKT	PCT	MKT	PCT	MKT	PCT	MKT
Significance levels if GHG production rates related to dry mass												
N	0.015	0.22	0.027	0.46	0.027	0.46	0.007	0.46	0.002	0.81	0.002	0.81
P	0.001	0.22	0.01	0.46	0.01	0.46	0.005	1	0.000	1	0.000	1
Significance levels if GHG production rates related to carbon content												
N	0.018	0.22	0.027	0.46	0.027	0.46	0.003	0.46	0.001	0.81	0.001	0.81
P	0.001	0.22	0.009	0.46	0.009	0.46	0.002	1	0.000	1	0.000	1

Table 6. Estimation of annual methane production due to decomposition of fresh aboveground plant litter on the basis of annual biomass production determined for sampled rewetted fens in the River Peene valley (Steffenhagen et al. 2012, Zak et al. 2014) and the daily methane production potential determined under lab conditions (see section 2.4). The latter values are based on initial dry mass.

Dominik Zak 3/16/15 10:17 AM
Kommentar: As requested by referee comment **R#2-5** we denoted that calculation is based on initial mass.

Plant species	in-situ annual biomass production kg dry mass/ha	Experimental determined daily methane production g CH_4/kg dry mass	annual methane production potential kg CH_4/ha
Phalaris arundinacea	6500	0.013	30
Ceratophyllum demersum	1000	0.404	147
Typha latifolia	12100	0.011	47
Phragmitis australis	16600	0.041	249
Carex riparia	7700	0.006	16



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3 Figure 1. The sampling sites located in north-eastern Germany.

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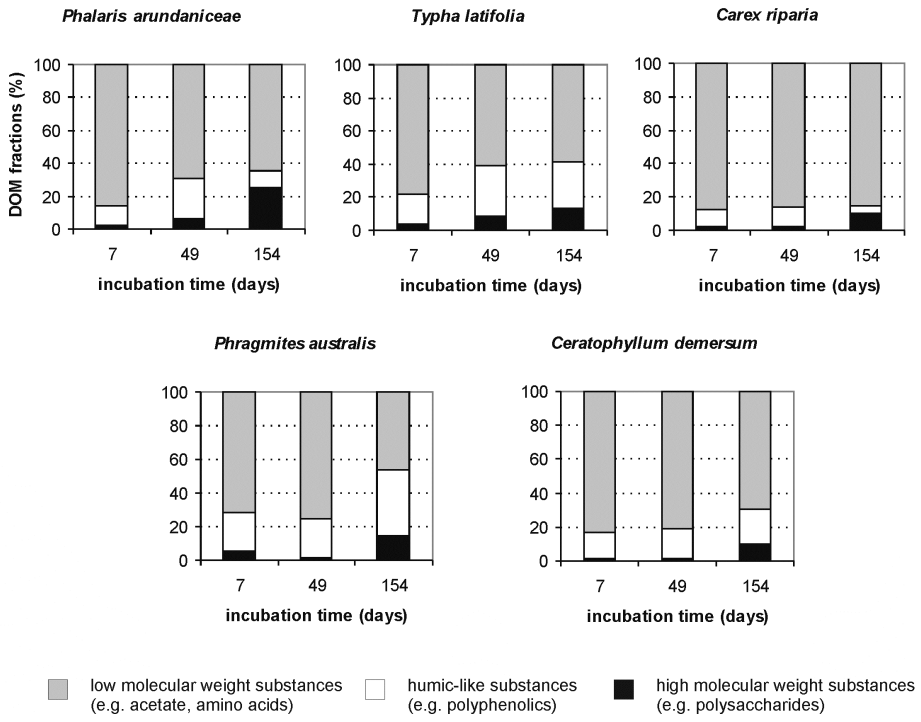

Figure 2. Composition of dissolved organic matter (DOM) in the water phase for all incubated plant species determined by liquid size-exclusion chromatography in combination with organic carbon detection (mean, n = 3).

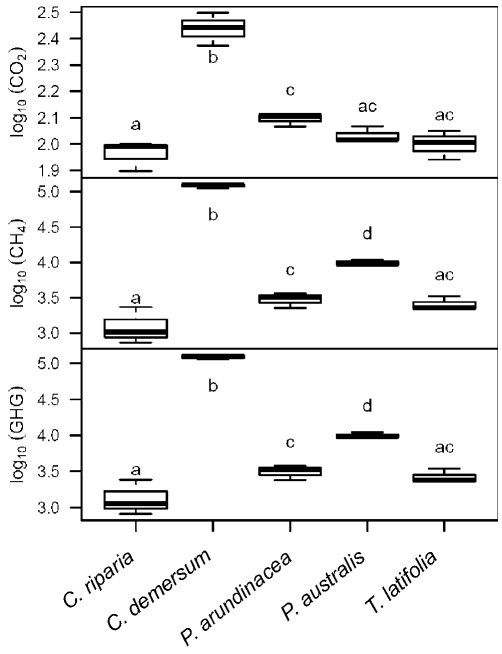

Figure 3. Boxplots of gas emission rates (mg CO_2-C/ g C; µg CH_4-C/ g C) based on initial carbon content of each species. Whiskers represent Min and Max values. Different letters indicate significant differences between species ($p < 0.05$).

Dominik Zak 3/16/15 10:18 AM
Kommentar: As requested by referee comment **R#2-5** we denoted that calculation is based on initial mass.

Dominik Zak 3/16/15 10:27 AM
Kommentar: To answer **R#2-7** we denoted Min and Max values here.

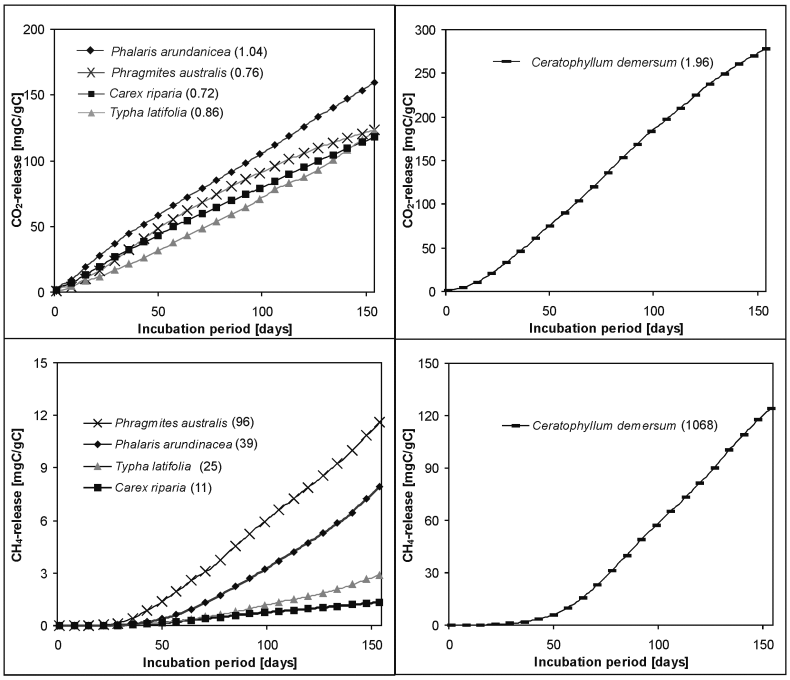

Figure 4. Cumulative release of CO_2 and CH_4 from different incubated plant material under submerged conditions over an incubation period over 154 d. The daily C-normalized gaseous C release is given in brackets (zero order rate constant: k(CO_2) / mgC/d gC, k(CH_4) / µgC/d gC calculated for day 40 to 154). Values are related to initial carbon contents and given as means (n = 3). For better visualisation the cumulative values after always seven days are highlighted by symbols.

Dominik Zak 3/16/15 10:19 AM
Kommentar: As requested by referee comment **R#2-5** we denoted that calculation is based on initial mass.