Title: Snags as straws? Quantifying the role of standing dead vegetation in the atmospheric flux of methane from wetlands.

Summary of August 2014 Pilot Project: In the early 2000s, the Great Dismal Swamp Restoration Bank, LLC began restoring the former Timberlake Farms in Tyrrell County, North Carolina (35°54'22"N, 76°09'25"W) for the purpose of adding the site to their mitigation bank portfolio. This ca. 1500 ha site (herein referred to as TOWeR: the Timberlake Observatory for Wetland Restoration) consists of 420 ha of mature forested wetland that was never under crop rotation, 787 ha of forested wetland, 57.2 ha of drained shrub-scrub pocosin, and 440 ha of former agricultural fields that are undergoing wetland restoration (Ardón et al., 2010).

After the last harvest in 2004, restoration of the site began with four primary goals in mind: 1) restore wetland hydrology, 2) restore the headwater streams, 3) restore the riverine wetland ecosystem, and 4) re-connect the hydrologically separated western forest to the site (Needham, 2006). Restoration began by filling 33 miles of vee-ditches that were used to drain the site while under crop rotation, plugging sections of the drainage canal, and creating a zone of preferential water flow per Army Corps of Engineers requirements. The next phase in the project consisted of restoring the vegetation community by planting 750,000 seedlings of a variety of species in appropriate locations based on habitat type: stream, riverine, and non-riverine. Finally, in 2007, the pump station, located at the northern end of the site, was disabled, restoring the historical hydrology to the site; it has since been removed.

With the onset of hydrologic restoration, the wetland began to flood. Several areas within the site that were not previously farmed were transformed into landscapes that look like ghost forests (Figure 1). The trees that were growing in these areas are not well adapted to the waterlogged soil created under flooded conditions; and, as they died, and left behind dead standing trunks (snags). On a trip to TOWeR in early 2012, our research group began to wonder if these standing snags could act as straws in the ground, serving as a direct pathway for the atmospheric flux of methane and other soil-borne greenhouse gases, thus bypassing the attenuating role of soil (van der Nat et al., 1998) and aquatic (Heilman and Carlton, 2001) guilds of microorganisms. (The effects of this direct pathway are herein termed the piping effect.)

Figure 1. A representative Ghost Forest landscape at TOWeR.

Wetlands have long been recognized as the single largest source in the annual flux of methane (CH_4) to the atmosphere (Ciais et al., 2013). But, the contribution of vegetation to this flux has historically received little attention. A recent review of the role of vegetation in the annual flux of methane to the atmosphere (Carmichael et al., 2014) indicates that vegetation may represent up to 22% (32-143 Tg CH_4 yr^{-1}) of this flux via both direct and indirect pathways. Though there is a large degree of uncertainty attached to this range of values, methane transport through wetland vegetation is likely to contribute significantly to this estimate. Prior research, as reviewed in Carmichael et al. (2014), has established the roles of 1) live herbaceous and woody vegetation and 2) dead herbaceous vegetation in atmospheric methane flux from wetlands. However, the role of dead woody vegetation in the annual flux of methane to the atmosphere has not yet been resolved.

In the summer 2014 field season, a small-scale (n=20) pilot project was conducted to determine if this pathway was worthy of further investigation. In August, two sites containing standing snags (Figure 2) were selected within the TOWeR property. Standing water depth at the sites ranged from 0.2-0.45 m, and tree diameter at breast height (DBH) values of the 20 trees measured ranged from 0.06-0.18 m. Trunk airspace and the air immediately outside of each trunk were sampled at breast height (to have a standardized height on each snag) and at water level (the hypothesized zone of maximum methane concentration) following a protocol outlined by Covey et al. (2012). Two heights per snag were sampled as studies from living trees indicate that methane flux decreases as trunk height increases (Pangala et al., 2012). Gas samples were analyzed via chromatography at the Duke University River Center for CH_4, CO_2, and N_2O within one week of collection. These samples yielded point-in-time concentrations for all three of these greenhouse gases, which were compared via statistical analysis. We were interested in determining if the concentration of a gas increased within the trunk airspace relative to the air outside, a possible indication of a piping effect.

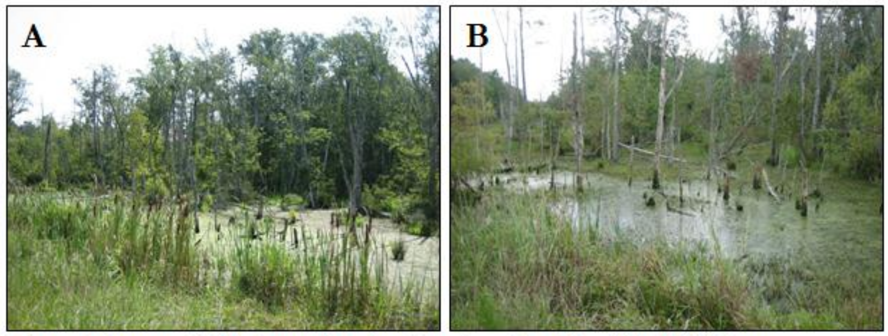

Figure 2. Summer 2014 pilot project sites. Two sites, A and B, within TOWeR were selected for sampling. Trunk airspace samples were collected from 10 trees per site.

There were no significant differences in gas concentrations in the trunk airspace compared to the air outside of the snag at breast height (Figure 3). However, for both CH_4 and CO_2, trunk airspace concentrations were significantly elevated at water level compared to the air immediately outside (Figure 4). In the case of CH_4, several of these samples were above the detection limit of 200 ppm. Additionally, for CH_4, concentrations were significantly elevated in trunk airspace at water level when compared to breast height (Figures 3 and 4). It is important to note that, in several trees, the wood at water level was damp/wet, which begs the question of whether the elevated CH_4 concentrations were a result of *in-situ* methanogenesis via decomposition, or if this was evidence of a piping effect. Interestingly, though, there were 2 dry samples at water level where the concentration of CH_4 in the trunk airspace was an order of magnitude higher (12× and 24× respectively) than the air immediately outside, indicating that the piping effect may at least play a role in the elevated CH_4 concentrations within trunk airspace, though more work is needed to confirm this assertion. We were excited about these initial results and are applying for the Garden Club of America's Wetlands Scholarship for the purpose of conducting a more definitive version of this project for the summer 2015 field season.

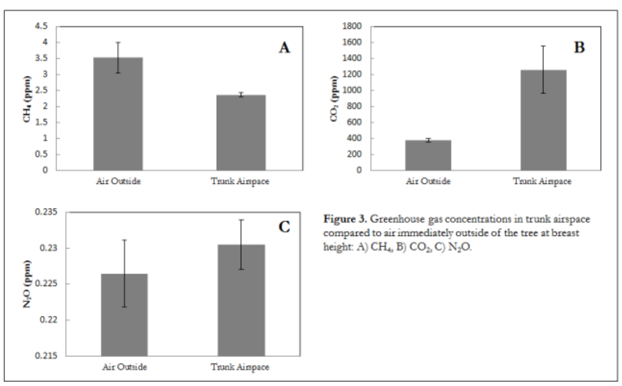

Figure 3. Greenhouse gas concentrations in trunk airspace compared to air immediately outside of the tree at breast height: A) CH_4, B) CO_2, C) N_2O.

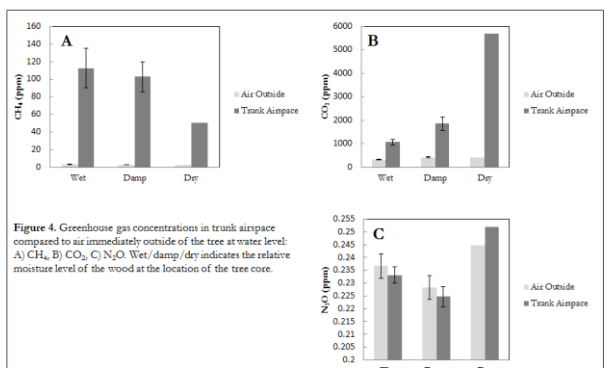

Figure 4. Greenhouse gas concentrations in trunk airspace compared to air immediately outside of the tree at water level: A) CH_4, B) CO_2, C) N_2O. Wet/damp/dry indicates the relative moisture level of the wood at the location of the tree core.

Statement of Proposed Work: Currently, knowledge regarding the role of wetland vegetation in methane flux to the atmosphere is constrained to live vegetation. The August 2014 pilot study indicated that dead vegetation (standing snags) may be an important unrecognized source of the atmospheric flux of this potent greenhouse gas. We propose an expanded study to investigate this phenomenon and quantitatively determine the role of standing dead vegetation in the atmospheric flux of methane from wetlands.

Quantifying methane flux from standing snags: A static chamber approach will be applied (Pangala et al., 2013; Pangala et al., 2012) to measure snag-atmosphere gas fluxes from 10 trees in 2 permanently inundated plots at TOWeR. Chambers will be constructed using clear Perspex® sheets following the design of Pangala et al. (2012) and deployed on trees at three height intervals, ranging from 0-30 cm (at water level) to 100-137 cm (at breast height, a standardized measurement). Gas samples will be collected in triplicate at 0, 5, 20, 50, 60, and 80 minutes in pre-evacuated 12 mL Exetainer vials and analyzed via chromatography at the Duke University River Center for CH_4, CO_2, and N_2O within one week. These data will reveal whether the methane concentrations found in trunk airspace remain contained within the trunk or escape to the atmosphere as a snag-based flux.

Quantifying methane flux from live trees: The approach described above will also be utilized to quantify the plant-atmosphere flux of methane from the trunks of 10 live bald cypress (*Taxodium distichum*) trees. These data will allow us to compare the relative roles of live and dead trees in the atmospheric flux of methane from wetlands.

Quantifying methane flux from the water-atmosphere interface: To provide a baseline for comparison, we also request funding to quantify the water-atmosphere gas fluxes from static chambers located next to each of the 20 trees sampled above. Chambers will be designed following the approach of Helton et al. (2014), which has been successfully utilized at TOWeR. Gas samples will be collected in triplicate at 0 min, 8 hr, and 24 hr and analyzed via chromatography at the Duke University River Center for CH_4, CO_2, and N_2O within one week. These data will be helpful in delineating the relative importance of the plant- and water-atmosphere pathways of methane flux from permanently inundated wetlands.

Elucidating the relative roles of in-situ decomposition versus the piping effect in methane flux from standing snags: Based on the design of the August 2014 pilot project, we were unable to determine the relative role of *in-situ* woody tissue decomposition versus a piping effect in the point-in-time methane concentrations within the standing snags. Therefore, as a proof of concept, we plan on installing 10 packed PVC columns in the ground next to each of the standing snags described above. These columns will be packed with a material that is equivalent to the density of the wood at the site and open at the top to the atmosphere. Sealed sampling septa will be permanently installed at three heights on the column (at water level, at 68.5 cm, and at 137 cm). We will utilize the point-in-time sampling method described above and inspired by Covey et al. (2012) to collect trunk airspace gas samples in triplicate at multiple time points throughout the day. In addition, we plan a laboratory-based anaerobic incubation of woody tissue sampled at water level from each snag (n=10). Woody tissue samples will be harvested in the field and incubated anaerobically from a modified protocol derived from Covey et al. (2012) and Yavitt et al. (1988). With the recognition that disturbance of methanogenic communities can impact methane production, each incubation will be sampled in triplicate daily over a period of a week to determine the role of *in-situ* decomposition in the methane concentrations observed in the August 2014 trunk airspace samples. When combined, these data will provide valuable information regarding the relative importance of decomposition and the piping effect as the source of elevated methane concentrations within the trunk airspace relative to air immediately outside of the trunk.

Additional ecological and environmental measurements: Additional ecological data to be collected include the species of each snag (woody tissue anatomical identification performed at North Carolina State

University), the density of the wood, and DBH. We will also measure water level, pH, and conductivity at each tree on the day of measurement. Water temperature, wind speed, and barometric pressure will be measured at the time of each gas sampling event. Permanently installed HOBO dataloggers at the site will log air temperature and relative humidity over the duration of the study period.

Benefit to Coastal Wetlands: If successful, this research will identify and quantify a novel pathway in the annual flux of methane to the atmosphere, moving us one step closer to closing the gap in the global methane budget. In addition, this flux due to the piping effect will be compared to that from both live vegetation and the air-water interface, thus providing a more comprehensive estimate of the role of vegetation in the atmospheric flux of methane from wetland systems. From a conservation perspective, a more comprehensive understanding of the pathways of methane flux from freshwater coastal wetlands will aid in management decisions for greenhouse gas emissions that affect both intact habitats and restoration efforts in coastal regions.

Budget: We request funding from the Garden Club of America for the analysis of gas samples at the Duke University River Center (2015 pricing, $2.94 per sample or standard). Additional funding will be obtained from internal sources for travel, and we have several proposals currently in review to fund materials and supplies for chamber construction, gas sampling, woody tissue incubations, and equipment.

Sample Category	**Price**	**Justification**
Snag-atmosphere flux	1587.60	10 snags at 3 heights sampled in triplicate at 6 time intervals.
Live tree-atmosphere flux	1587.60	10 trees at 3 heights sampled in triplicate at 6 time intervals.
Water-atmosphere flux	529.20	20 chambers sampled in triplicate at 3 time intervals.
PVC pipe proof of concept	529.20	10 pipes at 3 heights sampled in triplicate at 2 time intervals.
Woody tissue incubations	705.60	10 cores sampled in triplicate daily over a one week period.
Mileage reimbursement for transportation	92.40	One trip to the Duke River Center in Durham, NC (165 miles round trip) for sample analysis reimbursed at the University mileage rate of $0.56/mile.
Total Budget **Total Request from the Garden Club of America**	**$5031.60** **$5000**	

Broader Impacts: Planned outreach will occur at many levels, from within the local community to attendance at a national meeting. Within the department, I will give a talk in the Fall 2015 EcoLunch series that will be a follow-up to the talk I gave in the Fall 2014 series describing the August 2014 pilot study and proposed expansion of the project. Within the community, I will capitalize on my connection with the Wake Forest University Graduate School/Winston-Salem Forsyth County Schools Partnership and develop a lesson for middle school students on global climate change and associated coastal issues, which will include data from my research. At the state/regional level, this project will be presented (at a minimum) at one of the following conferences in 2016: the North Carolina Academy of Science General Meeting, the 3rd Annual Southeastern Biogeochemistry

Symposium (Georgia Institute of Technology), or the Association of Southeastern Biologists General Meeting. Finally, if possible, the work will be presented at the 2015 American Geophysical Union Fall Meeting.

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