Biogeochemistry (2009) 92:177–181
DOI 10.1007/s10533-008-9268-6

Contamination effects on soil density fractions from high N or C content sodium polytungstate

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Received: 25 March 2008 / Accepted: 8 October 2008 / Published online: 11 December 2008

Abstract Sodium polytungstate (SPT) is currently the material of choice for soil density fractionation (DF). We recently detected high levels of N in several types of commercially available SPT (0.74–1.4 mg g^{-1}), raising a concern that undesirable chemical effects on soils may occur during the DF procedure. To address this concern, we conducted two experiments to examine effects of SPT on C and N in the resulting soil fractions. First we suspended A-horizon material from three soil types of greatly differing mineralogy for 24 h in solutions containing three types of commercially obtained SPT and commercial SPT that had been passed through cation exchange resin columns. We compared %C, %N, ^{15}N and ^{13}C values in treated and untreated soils. We also spiked SPT with tracer-level $^{15}NH_4^+$ to measure potential NH_4^+ absorption by the soil fractions. Results suggest that the N-rich commercially available SPT can have a considerable effect on δ^{15}N values, likely due to the presence of ^{15}N-enriched NH_4^+ in the SPT. In one of our soils, ^{15}N enrichment of 3‰ was observed associated with overnight soaking in N-rich SPT (0.74 mg g^{-1}). By contrast, when using SPT with low levels of N (0.05 mg g^{-1}), no significant changes in ^{15}N were observed. The remaining soil (after suspension and rinsing) was similar in %C, %N, ^{15}N and ^{13}C to the untreated bulk soil, suggesting that suspension of soil in SPT with low N levels purchased from the manufacturer or else through treatment with cation exchange resins does not greatly alter these variables. Low-N SPT is available commercially although it must be specifically requested from the manufacturer and is currently more expensive to purchase. Our results confirm that SPT tested and known to be low in C and N (<0.06 mg g^{-1}) does not adversely contaminate soils during the soil density fractionation procedure. If using newly purchased or recycled SPT with higher N or C levels than this, we recommend thorough testing for possible contamination effects prior to use. However we caution against using SPT that contains N or C levels >0.5 mg g^{-1}.

Keywords Soil organic matter dynamics · Stable isotope ratios · Carbon · Nitrogen · Sodium polytungstate · SPT · Density fractionation · Natural abundance of ^{15}N and ^{13}C

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Introduction

Modelers and soil biogeochemists have long searched for techniques to identify meaningful pools of soil organic matter (SOM) that differ in mean turnover time, chemical composition, or both. Until these pools can be identified and separated, we cannot advance our understanding of those processes that cause changes in SOM stabilization and destabilization. Density fractionation is a widely used physical fractionation technique in SOM research (Baisden et al. 2002; Kramer et al. 2003; Sollins et al. 2006; Crow et al. 2007; Kaiser and Guggenberger 2007; Basile-Doelsch et al. 2007), and sequential density fractionation at a series of densities from 1.65 to 2.65 g cm^{-3} has been shown to offer a practical means for separating soils into fractions that differ with respect to the composition and dynamics of their organic content (Sollins et al. 2006). Because it is non-toxic, highly soluble, and can yield very dense solutions ($\sim$3 g cm^{-3}), sodium polytungstate (SPT) is currently the compound of choice for the density fractionation procedure. However, we recently detected high N levels in commercially purchased SOMETU [Berlin, Germany] and GeoLiquids [Prospect Heights, IL, USA] SPT (0.74, 1.4 mg g^{-1}, respectively), resulting from changes in EU regulations for the manufacturing of this chemical. Concern over both C and N contamination effects prompted us to compare four types of SPT from two manufacturers across a range of densities (Fig. 1). We also checked whether the density fractionation technique itself might be preferentially solubilizing C relative to N or ^{13}C and ^{15}N relative to ^{12}C and ^{14}N, causing artifacts in the values for soil fractions.

Methods

We conducted two experiments to examine potential contamination effects. First, we suspended three soils in four types of SPT across a range of solution densities (1.65–2.55 g cm^{-3}) and water over a 24-h period. The four types of SPT tested were 'old' circa 2001 SOMETU SPT, 'new' circa 2006 SOMETU SPT [Berlin, Germany], commercially available GeoLiquids SPT [Chicago, USA] and resin-column filtered GeoLiquids SPT. Resin-filtered SPT was prepared using the procedure of Six et al. (1999). For this portion of the experiment, we also tried using hydrogen peroxide with heating to 100°C to oxidize DOC in the recycled SPT using 40 ml of SPT at a density of 2.2 g cm^{-3}. Different vials received (1) 4 ml of DI water; (2) 2 ml of H_2O_2 and 2 ml DI; (3) 4 ml of H_2O_2; and (4) 2 ml H_2O_2 followed by heating and an additional 2 ml H_2O_2 with heating. The control solution (SPT with only water added) had a C content of 0.09 mg g^{-1}. The C and N content of each SPT type was determined using a Shimadzu TOC-TN analyzer. To reduce the salt content of samples, which can be problematic for a TOC-TN analyzer, we diluted SPT samples in water using a dilution ratio of 5 g SPT: 100 g H_2O.

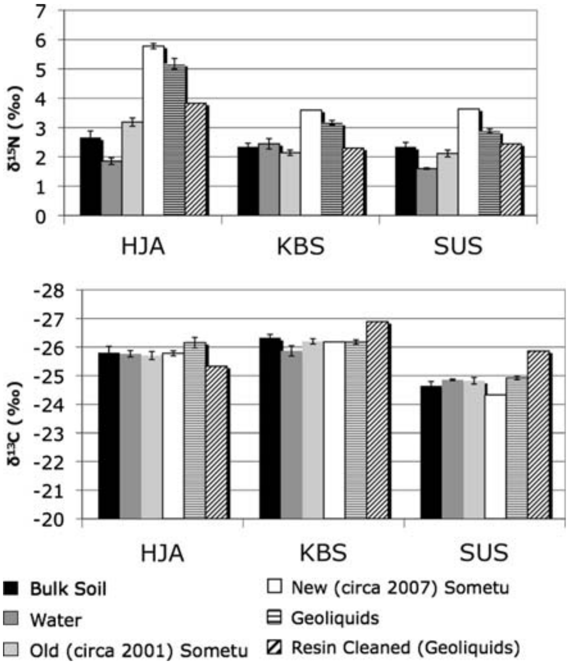

Fig. 1 Effects on ^{15}N and ^{13}C of suspending three soils in four types of commercially available SPT: Old Process (circa 2001) SOMETU (0.05 mg N g^{-1}), New Process (circa 2007) SOMETU (0.74 mg N g^{-1}), and new GeoLiquids (1.4 mg N g^{-1}) and resin-cleaned GeoLiquids SPT (0.34 mg N g^{-1}). Data are averages across SPT solutions of three densities: 1.65, 2.4 and 2.6 g cm^{-3}. *Error bars* are shown for replication > 2

The soil types we tested included an Oxisol from Puerto Rico (SUS), an Alfisol from the Kellog Biological Station, Michigan, USA (KBS), and an Inceptisol (volcanically derived) from the H. J. Andrews Experimental Forest, Oregon, USA (HJA). Between 4–5 g A-horizon material was added to each solution treatment (depending on the amount of soil material we had) using a 1:5 soil mass:solution volume ratio in duplicate. Bulk %C, %N, and ^{15}N and ^{13}C stable isotope values were compared before and after suspension in the solutions after thoroughly rinsing the soil to remove SPT. To minimize soil particle loss during rinsing, we washed samples in 250-ml conical centrifuge tubes. After centrifugation, all material that floated at a given density (referred to here as light fraction) was decanted into a separate 250-ml centrifuge tube. Water was then added to the light-fraction centrifuge tube and any floating material was aspirated and rinsed on GF/F filters [Whatman Incorporated, Clifton, New Jersey] a minimum of four times with 235 ml DI water. The remaining soil was dried (40°C) in the centrifuge tube. Light and heavy fractions were then physically recombined, weighed, and prepared for C, N, ^{13}C and ^{15}N analysis. Dry samples (<2 mm) were ground finely with a zirconium mortar and pestle, and loaded into tin boats. C, N, δ^{13}C and δ^{15}N were measured with a coupled continuous-flow elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) system. Samples were analyzed with a Carlo-Erba model 1108 EA interfaced to a Thermo-Finnigan Delta Plus XP IRMS. Analytical precision of in-house standards, which had been calibrated using international standards, was typically better that 0.2‰ for both δ^{13}C and δ^{15}N.

In our second experiment, we assessed the ability of different density fractions of the andic Inceptisol (HJA) and the Oxisol (SUS) to sorb ammonium N from SPT solutions by comparing the ^{15}N concentration in soils soaked in a ^{15}NH$_4$Cl-spiked SPT solution with that in an unspiked SPT control. We used ^{15}NH$_4$Cl because we felt that NH$_4{}^{+}$-N was most likely to be the form of N to sorb to soils, specifically to the soils with high clay content or CEC, and we wanted to test this mechanism as the source for possible contamination by SPT, even though SPT solutions have high Na^{+} concentrations. The SPT used for the spiking experiment was "new" high-N SOMETU SPT (0.74 mg g^{-1}), and it was spiked with 3 μg ^{15}N g^{-1} SPT. To determine the amount of sorbed ^{15}NH$_4$Cl to soil samples we measured ^{15}N content above natural abundance levels, using a PDZ Europa 20–20 isotope ratio mass spectrometer equipped with a Europa Roboprep elemental analyzer.

Results and discussion

We found that the SPT available in 2007 from commercial sources (SOMETU and GeoLiquids) contains high levels of N (0.74 mg g^{-1}, 1.4 mg g^{-1}, respectively) and can have a considerable contamination effect, most notably on δ^{15}N values (Fig. 1) and showed a consistent but slight increase in soil N content (Fig. 2). The C:N ratio of this SPT was very low (0.09), indicating that the N is largely inorganic. Our ^{15}NH$_4$Cl soaking experiment showed that both HJA and SUS soils absorbed on average 0.76 ± 0.12 mg g^{-1} of labeled ^{15}NH$_4{}^{+}$ from solution. N sorption to soil increased from 0.46 mg g^{-1} in a low (1.65 g cm^{-3}) density solution up to 0.99 mg g^{-1} at high (2.55 g cm^{-3}) density. These amounts were consistent with N sorption observed in the N-rich SPT soaking experiment (0.16–2.12 mg). The C content of the SPT we tested ranged from 0.09 mg g^{-1} for Old SOMETU, to 0.06 and 0.05 mg g^{-1} for GeoLiquids and New SOMETU, respectively. For all our experiments, soil C and ^{13}C appeared to be little affected by any of the

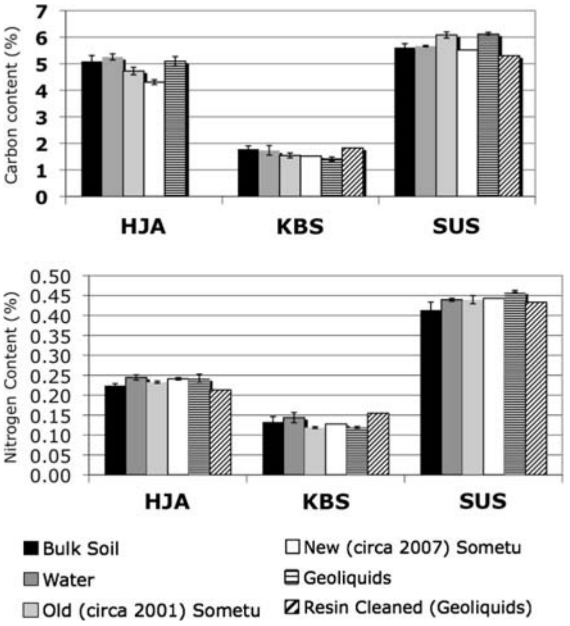

Fig. 2 Effects on N and C content (%) of suspending three soils in four types of commercially available SPT: Old Process (circa 2001) SOMETU (0.05 mg N g^{-1}), New Process (circa 2007) SOMETU (0.74 mg N g^{-1}), and new GeoLiquids (1.4 mg N g^{-1}) and resin cleaned GeoLiquids SPT (0.34 mg N g^{-1}). Data are averages across SPT solutions of three densities: 1.65, 2.4 and 2.6 g cm^{-3}. *Error bars* are shown for replication > 2

commercial SPT we tested (Figs. 1, 2). Resin-cleaned SPT produced inconsistent results, with ^{13}C increasing in two soils and decreasing in one soil compared to bulk soil values. In two of the soils, overnight soaking in N-rich SPT increased soil N concentration by as much as 10% and in all soils we observed ^{15}N enrichment (as high as 3‰) (Figs. 1, 2). Figure 3 shows that the extent of ^{15}N enrichment after soaking in N-rich SPT was greatest at higher densities (2.4–2.6 g cm^{-3}) but that a contamination effect was still apparent at lower densities (1.65 g cm^{-3}). Light and heavy density soil fractions each individually showed a pattern of ^{15}N enrichment after suspension in the N-rich SPT, but we used the comparison between recombined ^{15}N values and bulk soil ^{15}N values as our overall measure of contamination. While New SOMETU SPT N levels were lower than GeoLiquids (0.74 vs. 1.4 mg g^{-1}) the ^{15}N contamination effect was greater from New SOMETU SPT, suggesting that the ^{15}N values of the New SOMETU SPT were greater. Unfortunately, we did not measure ^{15}N values of the new or old SOMETU SPT because of concern that the salts may damage our mass spectrometer. We did however measure ^{15}N for GeoLiquids SPT, which was 19‰. Our results suggest that both the ^{15}N value and the amount of N in the SPT influence the extent of contamination to soil. By contrast when using low-N SPT (<0.06 mg g^{-1}), in this case SOMETU SPT purchased before 2002, we noted only slight changes in ^{13}C, ^{15}N, C, or N concentrations (Figs. 1, 2). Similarly, N-rich SPT that was cleaned by passing through cation-exchange resins (Six et al. 1999) eliminated the increase in ^{15}N that we found for the same SPT that had not been passed through resins (Fig. 1). Although the N content of the recycled SPT decreased from 1.4 to 0.34 mg g^{-1} the N levels were still quite high.

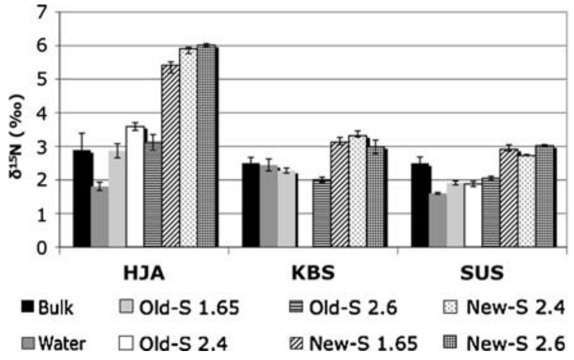

Fig. 3 Detailed density effects on ^{15}N and ^{13}C of suspending three soils in two types of commercially available SPT: Old Process (circa 2001) SOMETU (0.05 mg N g^{-1}), New Process (circa 2007) SOMETU (0.74 mg N g^{-1}). Data are SPT solutions of three densities: 1.65, 2.4 and 2.6 g cm^{-3}. *Error bars* are shown for replication > 2

We also attempted to reduce the C content of SPT by passing used SPT through activated charcoal, following the method of Six et al. (1999), but in our experiments, C content actually increased from 0.06 to 0.12 mg g^{-1} with passage through charcoal columns, possibly due to ultra-fine charcoal particles filtering through the glass wool filters at the base of all columns. This discrepancy is perplexing, and may be due to differences in batches of activated charcoal that are commercially available. Further reduction of C levels in SPT to 0.04 mg g^{-1} (a reduction of over 50%) was found to be possible using the H_2O_2 treatment. However during the H_2O_2 procedure, a white powder precipitated and the solution turned yellow, suggesting that the SPT had been made less stable, and therefore we do not recommend chemical oxidation to remove organic carbon.

On average, 0.23 ± 0.03 g [mean ± 1 SE] of soil was lost as a result of suspension, filtration and rinsing, which is a typical soil amount lost in a properly performed DF procedure. Yet bulk soil values for C, N, ^{15}N, and ^{13}C were similar to those for soil after 24-h suspension in clean SPT ("Old SOMETU") (Figs. 1, 2) suggesting that density fractionation, when performed with clean SPT, does not significantly alter ^{13}C, ^{15}N, C, or N concentrations in any of the soils we examined, despite appreciable soil loss (in this case as high 6% since we only used between 4 and 5 g of starting material). We also found that, for two of the three soils, suspension of soil in water only caused slight ^{15}N depletion. This depletion could be due to preferential desorption of ^{15}N, which did not happen with suspension in higher ionic-strength SPT (Evans et al. 1988). When employing the density fractionation procedure we reiterate the recommendation of others (Crow et al. 2007) that any desired chemical measurements be made on all density fractions and on the bulk soil, rather than attempting to calculate values for any fractions by difference. Mathematically recombined values from each of these fractions (using the relative proportions from each) should be routinely compared with bulk soil values during the

DF procedure to ensure no undesirable artifacts occur.

The mathematical formula for recombining ^{15}N values, was based on:

$$\delta^{15}\mathrm{N\ Mathematically\ recombined\ fraction} = \frac{\mathrm{LF\ wt(g)}}{\mathrm{(LF+HF)\ wt(g)}} \times \delta^{15}\mathrm{N\ LF} + \frac{\mathrm{HF\ wt(g)}}{\mathrm{(LF+HF)\ wt(g)}} \times \delta^{15}\mathrm{N\ HF}$$

where LF is the Light Fraction and HF is the Heavy Fraction.

Given that SPT manufacturing protocols changed within the last few years, resulting in high N levels (0.74–1.4 mg g^{-1}), our results suggest the need to use newly available low-N and low-C SPT in order to avoid unintended alteration of C, N, ^{15}N or ^{13}C values during density fractionation. We have been working with both of the major SPT manufacturers (SOMETU and GeoLiquids) so that low C and N content SPT can again be purchased, although currently (as of November 2008) low C and N products (0.074 and 0.05 mg g^{-1}, respectively) are available only through special request; new manufacturing techniques for commercial production of low C and N SPT are still being developed. As part of that process, we are asking them not only to report C and N levels, but to also measure and report ^{15}N and ^{13}C content of the SPT. If purchasing SPT through either GeoLiquids or SOMETU we recommend specifically asking for this 0.05 mg g^{-1} N content product, and confirming with the manufacturer that C and N content of each new lot has been measured. Our expectation is that eventually this low-N product will become a standard commercial SPT product for both SOMETU and GeoLiquids, with QC measures developed that guarantee low (<0.06 mg g^{-1}) C and N content when purchased.

A cost-effective alternative may be to use cation exchange resin-cleaned recycled SPT. However, because we found that resin-cleaned SPT still contained considerable N (0.34 mg g^{-1}) we recommend that any batch of recycled SPT still be tested for potential contamination effects on soil prior to use. Additionally C levels in recycled SPT may be considerably higher than commercial SPT C content (Six et al. 1999), therefore C contamination may be a real concern too. If working with recycled SPT or with SPT of unknown N content, the simplest procedure is to measure the N and C content of SPT solutions with a TOC-TN analyzer and to test potential contamination effects of SPT on the specific soil to be used by overnight soil soaking prior to use. The overnight soaking experiments we conducted are easy to implement and provide a way to rapidly evaluate potential SPT contamination effects during the DF procedure. Finally, because we found that SPT with N levels between 0.74 and 1.4 mg g^{-1} did contaminate the soils we tested, we caution against using either new or recycled SPT that contains N levels >0.5 mg g^{-1}.

Acknowledgments We thank Jonathan Giska and Russell Johnson for help with running the experiments. Funding for this work was provided by grants from the USDA CSREES NRI program (2007-03184 to Marc Kramer, 2002-35107-12249 to Kate Lajtha and 2005-35107-16336 to Phillip Sollins).

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