COMPARISON OF OXYGEN-BOMB COMBUSTION WITH STANDARD IGNITION TECHNIQUES FOR DETERMINING TOTAL ASH[1]

WILLIAM A. REINERS AND NORMA M. REINERS

Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire

Abstract. Ash was determined in a variety of plant and organic soil samples by oxygen-bomb combustion and by a simple muffle-furnace technique. The influence of using different drying temperatures was also assessed. Bomb combustion produced a systematic error of underestimate and a random error of variation between trials. These errors varied among the different types of material tested, but in general both types of error increased with the ash content of the material. The average systematic error of underestimate of ash content for all samples was 1.46%, which led to an error of 1.56% when adjusting caloric coefficients to an ash-free basis. Lower drying temperatures had an insignificant effect on adjusted caloric coefficients. Independent ash determinations are recommended for materials with ash content greater than 5% to restrict the error of adjusted coefficients below 1%. The muffle-furnace technique is recommended for close approximations of absolute ash content.

INTRODUCTION

Caloric data are most useful on a dry weight basis for converting biomass to energy equivalents, but for comparisons along taxonomic or ecologic lines ash-free values are needed (Cummins 1967). By eliminating the inorganic ash component better evaluation of the biochemical properties of materials, such as higher fat content, are possible. Also, ash-free values eliminate errors of contamination by inorganic soil particles (Golley 1961).

Many investigators have adjusted caloric values to an ash-free basis by measuring the residual ash remaining after combustion in the calorimeter (Long 1934, Golley 1961, 1969, Comita and Schindler 1963, Gorham and Sanger 1967, and Ovington and Lawrence 1967). Cummins (1967) stated that such ash determinations are highly variable and separate measurements are advisable. Recognizing this variation, some investigators have made separate measurements of ash for correction of caloric values to an ash-free basis (Richman 1958, Malone and Swartout 1969). Odum, Marshall, and Marples (1965) noted that calorimetry-derived ash values tended to be lower than ash values derived from muffle-furnace combustion, indicating a possible systematic source of error in addition to random variation. They did not show data, however.

We have made numerous caloric and inorganic nutrient determinations for a wide variety of plant materials as part of a study of forest energetics and nutrient cycling (Reiners and Reiners 1970). These analyses have provided an opportunity to compare ash determination by oxygen-bomb combustion with dry-ash techniques. The objective of this paper is to assess the error caused by adjusting caloric coefficients to an ash-free basis through the use of residual ash weights derived by standard colorimetry.

[1] Received May 13, 1970; accepted February 24, 1971.

METHODS

One-hundred and thirty-nine samples from a variety of plant tissues and 15 organic soil samples were analyzed as described below by oxygen-bomb combustion and muffle-furnace techniques (see Table 1 for listing of tissues). Thirty-two representative plant-tissue samples and four organic soil samples were also analyzed by the char method (see below). All materials were dried for 48 hr at 55°C. This relatively low temperature was used to maintain a percentage moisture basis consistent with other forms of analysis followed in the ecosystem study. A drying temperature of 55°C was selected to minimize vaporization of volatile nitrogenous compounds which would lead to underestimates by nitrogen analyses. These volatiles also contributed to caloric content. The effects of different drying temperatures are discussed below. Materials were ground through a 20-mesh sieve in a Wiley mill for dry-ashing; subsamples were ground more finely through a 60-mesh sieve for calorimetry.

Oxygen-bomb combustion

Samples were pressed into pellets when necessary and redried for 16 hr at 55°C. As with all methods, dried samples were cooled in a desiccator before weighing. Approximately 1 g of material was ignited, except for those materials requiring less to achieve proper combustion. Samples were ignited at 20 atm oxygen in a Parr adiabatic bomb calorimeter according to the methods outlined by Parr (1960) including correction for fuse wire combustion and acid formation. Following ignition, obvious fragments of partially burned fuse wire were removed, and the capsule and ash were dried and weighed. Combustions were repeated until two caloric results varied by less than 100 g cal/g dry weight, representing a variation of 2.0% for a sample with an energy content of 5,000 cal/g. This usually required only two measure-

ments, and ash values presented here are means of these replicates. Average differences between ash estimate of these two measurements were slightly more than 10% of the mean ash value, and the maximum differences between replicates among the nine tissue types was 31%, indicating that ash determinations varied more widely than did energy determinations by this method.

Muffle furnace

The second method was part of routine dry-ashing for inorganic nutrient analysis. For convenience this method will be referred to as the muffle-furnace method. Approximately 1 g of material was redried for 16 hr at 55°C and weighed in a 40-ml fused-silica crucible. The crucibles were placed in a cold, Thermolyne model F-A1740 muffle furnace, and the temperature was raised during 1 hr to an average temperature of 575°C. We later found by independent calibration with a mercury-in-glass thermometer that, although the furnace's thermocouple control system equilibrated at 525°C, temperatures ranged from 525° near the door to 590° in the rear of the oven. The samples were held at 575° for 4 hr, then allowed to cool slowly. While still warm they were transferred to a desiccator for final cooling to room temperature, after which they were weighed. Samples were not replicated in these measurements so there was no measure of reproducibility for this method. Replication in the very similar char method described below, however, suggests an average difference between replicates of about 1.1% of the means.

Char method

The third method was designed specifically for ash measurement (Jackson 1958: 330) and was used to provide a highly standardized value for ash contents. The muffle furnace was also used in this technique, but for clarity it will be termed the char method. One-gram samples were redried at 100°C for 10 hr, weighed, saturated with 2 ml olive oil, charred over gas flame until smoking ceased, and then ignited in the muffle furnace. The samples were brought to temperature during 1 hr and held at 575°C for 45 min. After cooling, the ash was moistened with water, dried over a sand bath, reheated at 575°C for 1 hr, cooled, and reweighed. Samples were reheated in the muffle furnace until the ash reached constant weight. Two duplicates of each sample were run through this procedure giving an average difference between replicates of 1.1% of the means.

Drying tests

Since the drying temperature used in the char method was higher than that used for the bomb-combustion and muffle-furnace methods, two tests of the effects of drying temperature were made. Twenty-two samples representing major tissue types and the range of ash contents encountered were redried for 16 hr at 55°C, weighed, then dried at 100°C for 10 additional hours, and reweighed. The second test involved duplicates of the same 22 samples. These were also redried for 16 hr at 55°, weighed, but then held at 55°C until they reached constant weight before the final drying at 100°C for 10 hr.

RESULTS

Oxygen-bomb combustion versus muffle-furnace results

Bomb-combustion percentages were compared with muffle-furnace results by linear regression analysis. Individual regressions were computed on tissue types, and data were combined into a general regression (Table 1). All significance tests were made at the 0.95 confidence level by methods described in Steel

TABLE 1. Statistics for regressions of oxygen-bomb ash percentages (dependent variable) versus muffle-furnace estimates

Sample	Slope[a]	*Y* intercept	$\bar{x}$[b]	Range (%)	Sample (number)	Correlation coefficient	Standard error of estimate	Difference at $\bar{x}$ (%)
Branch wood and bark	*0.45±0.25[c]	0.74	2.22	1.3–3.1	15	0.73	0.23	−0.77
Wood	*0.53±0.16	0.45	2.41	1.1–4.0	15	0.89	0.20	−0.68
Tree roots	0.38±0.32	0.72	2.71	1.3–3.8	15	0.58	0.42	−0.96
Current twigs	0.66±0.32	0.48	3.72	2.4–5.5	14	0.79	0.51	−0.79
Cones, aments	0.58±0.25	−0.03	3.93	3.1–5.5	5	0.92	0.27	−0.98
Tree leaves	0.87±0.14	−0.78	7.10	5.0–12.2	15	0.97	0.48	−1.70
Bark	*0.65±0.06	0.30	7.77	2.4–16.9	22	0.98	0.51	−2.42
Herbaceous shoots	0.82±0.16	−0.38	8.67	3.5–13.9	38	0.86	1.14	−1.94
Organic soils	1.02±0.03	−2.08	12.66	15.9–53.9	15	0.999	0.69	−1.83
All plant material	*0.75±0.04	−0.04	5.68	1.3–16.9	139	0.96	0.76	−1.46
Combined data	0.96±0.02	−1.15	7.70	1.3–53.9	154	0.99	1.07	−1.46

*Asterisks indicate that the slopes are significantly different from a slope of 1.
[a]All slopes are significant at the .995 confidence level except those for wood and cones which were significant at the .975 level.
[b]$\bar{x}$ = mean of ash contents in the particular set as determined by the muffle-furnace method.
[c]Ninety-five per cent confidence limits.

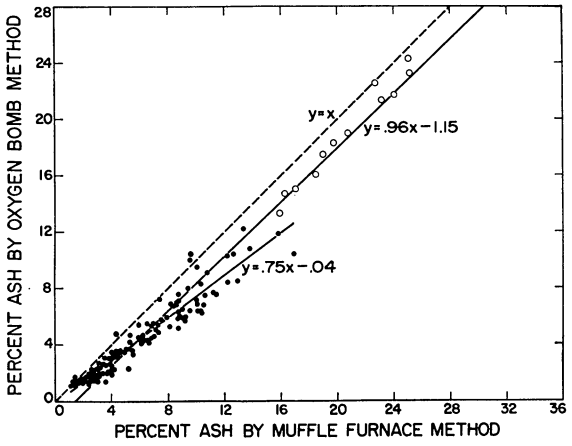

FIG. 1. Percentage ash by dry weight measured by the oxygen-bomb method plotted against percentage ash measured by the muffle-furnace method in comparison with a direct equivalence line (dashed line). Open circles represent organic soil samples, three of which are off-scale (42%, 53%, and 54%). The regression line labeled $y = .75x - .04$ was computed for all plant samples and is only extended over the range of those samples.

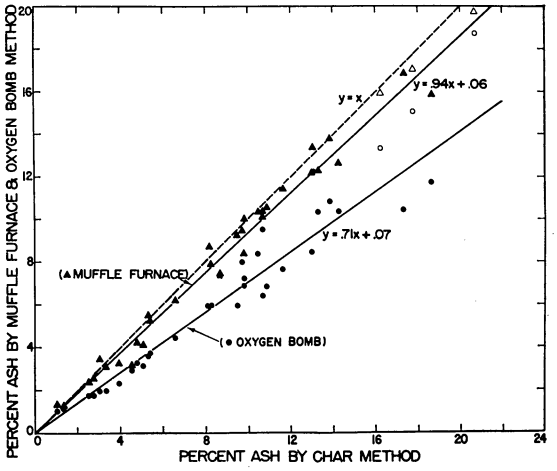

FIG. 2. Percentage ash by dry weight as determined by oxygen-bomb and muffle-furnace techniques plotted against percentage ash measured by the char method. Open symbols represent soil samples, one of which with 43% ash is off the x axis. Regression lines for both sets of data excluding soil data are shown in comparison with a direct equivalence line.

and Torrie (1960). Combined regressions for all plant tissues and for plant-plus-soil samples are graphed in Fig. 1.

As reported by Odum, Marshall, and Marples (1965), ash results based on bomb-combustion data consistently underestimated muffle-furnace results. The degree of underestimate generally increased with ash content (column 9, Table 1), but was especially high for bark samples and lower for organic soil samples. Average underestimates ranged from a low of 0.68% for wood with an average ash content of 2.41%, to a high of 2.42% for bark with an average ash content of 7.77%. An average difference of estimate was 1.46% based on all samples combined in one regression.

An examination of the results of the two methods indicates a sharp divergence beginning at about 1.5% ash and ending at about 4% ash. This was reflected in the very low regression slopes of low-ash tissues such as wood and bark, wood, and tree roots. Thereafter, slopes tended to approach unity (regression tended to parallel the equivalence line), but Y intercepts were less than zero. The bark regression continued to diverge more than the others in this ash range, whereas the organic soil regression almost exactly paralleled the equivalence line.

Comparison with char-method results

Samples were analyzed by the char method to compare bomb-combustion and muffle-furnace results with as near an approximation of absolute ash contents as possible (Fig. 2). Muffle-furnace results were only 0.5% lower than char values, on the average, a difference exceeded by the standard error of

TABLE 2. Statistics for regressions of oxygen-bomb- and muffle-furnace-derived ash percentages versus char-method values (regressions were calculated with and without organic soil samples for both sets of data)

Data	Slope[a]	Y intercept	$\bar{x}$	Range (%)	Sample (number)	Correlation coefficient	Standard error of estimate	Difference at $\bar{x}$
Oxygen-bomb data without soils	0.71±0.08	0.07	8.47	1.1–18.6	32	0.96	0.96	−2.38
Oxygen-bomb data with soils	0.91±0.06	−1.49	10.25	1.1–43.4	36	0.98	1.42	−0.92
Muffle-furnace data without soils	0.94±0.05	0.06	8.47	1.1–18.6	32	0.99	0.63	−0.51
Muffle-furnace data with soils	0.96±0.03	−0.10	10.25	1.1–43.4	36	0.99	0.61	1−0.41

[a]The slopes of all four regressions were significantly different from a slope of 1. Oxygen-bomb and muffle-furnace slopes were significantly different from each other, both in comparisons with, and without, organic soil samples. All slopes were significant at the 0.995 confidence level.

estimate (Table 2). This suggests that the relatively simple muffle-furnace technique closely approximates absolute ash values. Oxygen-bomb data were lower than char data by approximately the sum of the difference between oxygen-bomb and muffle-furnace, plus the difference between muffle-furnace and char results.

Organic soil results underestimated char values to a lesser degree than did plant-tissue results. To separate the influence of soil data on regressions, separate regressions with and without soil data were computed (Table 2). Organic soil data tended to parallel char data and because of their high ash values had a strong effect of bringing the slopes parallel with the equivalence line. As a result, when soil-sample data were combined with plant-tissue data, coincidences of estimates were much closer.

Drying tests

In the first drying test, samples lost an average of 2% in weight between the first redrying at 55°C and the second drying at 100°C. Loss was least with wood samples at 1.1% and greatest with organic soils at 3.2%.

In the second drying test, samples lost an average of 1% in weight between the first redrying at 55°C for 16 hr and constant weight, which was not achieved until 77 hr at that temperature. Average weight dropped further to an average of 2.7% of the weight at the end of the first 16 hr by the end of the 100°C drying period. Overall weight loss increased in the following order: wood, current twigs, herbaceous shoots, cones and aments, bark, tree leaves, organic soils.

Discussion

Bomb-combusion estimates of ash had two forms of error: a systematic error of underestimation and random error. The degree of systematic error depended on the nature of the material, especially its ash content (Table 1). Organic soils exhibited nearly constant underestimates over a broad range of ash content. The underestimate in plant materials, on the other hand, increased with increasing ash content. For example, the ash-free caloric coefficient of wood, with a dry weight coefficient of 4,800 cal/g, and with 2.41% ash, is 4,918. With an underestimate of ash of 0.68% (Table 1), the ash-free coefficient would be 4,884, an underestimate of 34 calories, or 0.69% of 4,918. For materials of higher ash content, the error is substantially greater. A bark sample with an ash content of 7.77% and dry weight caloric coefficient of 4,800 would adjust to 5,204. With an underestimate for ash of 2.42%, the ash-free coefficient would be 5,071, an underestimate of 133 calories, or 2.5% of 5,204.

A 2.5% error in determining ash-free caloric coefficients is probably near maximum since most plant materials have lower ash contents. Although this error only slightly exceeds the 2.2% variation permitted between calorimetric runs in this work, it will systematically cause a regular bias towards underestimation.

The systematic source of error might be accounted for with familiarization of the material tested, followed by application of corrective coefficients. The random source of error, however, is largely unpredictable and can be reduced only by the skill of the operator.

Although there is no evidence in our data that the amplitude of random error varies with the ash content of material, the effect of random error becomes more serious with increasing ash content. For example, with material of 2% ash and a dry weight caloric coefficient of 4,800, ash estimates varying by 10% lead to a trivial difference of 0.1% of the ash-free coefficient adjusted by the means of the two estimates. For material with 10% ash, however, a 10% difference between estimates leads to differences of 1.1% of the coefficient. These examples are based on the average difference between replicates. Obviously, in half of our data, differences were higher than 10%. On the other hand, the problem is partially mitigated by the use of means of two estimates.

According to these data, both the systematic error of underestimate and the random error become more serious with increasing ash content. To the extent that materials and techniques resembled ours, caloric data in the literature which have been adjusted with the residual ash resulting from bomb combustion underestimate true values by up to 2.5%. Although this error is small compared with 10% levels of error commonly accepted in ecological research, it can lead to large differences when caloric coefficients are multiplied by large biomass values to calculate standing crop in energy terms. Fortunately, such multiplication is usually done with caloric coefficients on a dry weight basis.

We recommend that decisions regarding the necessity of performing independent ash determinations for adjusting caloric coefficients be based chiefly on the range of ash content in the material to be tested. This decision may be guided by regressions in Table 1. In general, independent ash determinations are suggested for materials with above ca. 5% ash content to restrict the error of adjusted coefficients below ca. 1%. Independent estimates are recommended for all materials if the total ash in biomass is to be calculated.

When independent ash determinations are required, the muffle-furnace method is a simple technique for approximating absolute values. Muffle-furnace results were lower than char results by only

0.5% on the average (Table 2), an underestimate exceeded by the error of estimate (0.63%).

Drying at 55°C gave dry weight values about 2% higher than did drying at 100°C. It is not known what proportion of difference was contributed by water or by volatile organic losses. Such a difference would decrease the estimate of ash content by only 2%. As shown above, this variation is trivial for adjusting caloric coefficients. It should be considered, however, if ash percentages are to be multiplied by biomass to give total ash weights.

ACKNOWLEDGMENTS

This work grew out of research supported by National Science Foundation grants GB-3636 and GB-7148. The authors wish to thank Prof. Harold L. Allen, Dartmouth College, Prof. K. W. Cummins, Michigan State University, and Prof. E. Gorham, University of Minnesota, for their helpful comments.

LITERATURE CITED

Comita, W. G., and D. W. Schindler. 1963. Calorific values of microcrustacea. Science **140**: 1394–1395.

Cummins, K. W. 1967. Calorific equivalents for studies in ecological energetics. 2nd ed. Pymatuning Laboratory of Ecology, Pittsburgh, Pa. 52 p. (Mimeo.)

Golley, F. B. 1961. Energy values of some ecological materials. Ecology **42**: 581–584.

———. 1969. Caloric value of wet tropical forest vegetation. Ecology **50**: 517–519.

Gorham, E., and J. Sanger. 1967. Caloric values of organic matter in woodland, swamp, and lake soils. Ecology **48**: 492–494.

Jackson, M. L. 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. 498 p.

Long, F. L. 1934. Application of calorimetric methods to ecological research. Plant Physiol. **9**: 323–337.

Malone, C. R., and M. B. Swartout. 1969. Size, mass and caloric content of particulate organic matter in old-field and forest soils. Ecology **50**: 395–399.

Odum, E. P., S. G. Marshall, and T. G. Marples. 1965. The caloric content of migrating birds. Ecology **46**: 901–904.

Ovington, J. D., and D. B. Lawrence. 1967. Comparative chlorophyll and energy studies of prairie, savanna, oakwood, and maize field ecosystems. Ecology **48**: 515–524.

Parr Instrument Company. 1960. Oxygen calorimetry and combustion methods. Manual no. 130. Moline. Illinois. 56 p.

Reiners, W. A., and N. M. Reiners. 1970. Energy and nutrient dynamics in forest floors in three Minnesota forests. J. Ecol. **58**: 497–519.

Richman, S. 1958. The transformation of energy by *Daphnia pulex*. Ecol. Monogr. **28**: 273–291.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 p.