A Guide to Conducting Biochar Trials



Preparing lump charcoal for soil application in Honduras. Photo by J. Major.



Biochar field trial with maize in Aurora, NY USA. Photo by C. Hyland

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| | This document was reviewed by IBI's Science Advisory Committee. |
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| IB of g | I and Julie Major are not responsible for any prejudice caused by the application uidelines given in this manual. Figures and tables presented herein are not based on real data and are shown as fictitious examples only. |
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Introduction

Biochar has been shown to benefit crop growth and yield, and is a promising material for use in agriculture. However, as is the case for any soil amendment, its efficacy must be shown in a variety of cropping systems, and at this time (2009) optimal application rates have yet to be determined. Also, many groups and individuals coming from a variety of backgrounds are interested in testing biochar. This is why the International Biochar Initiative (IBI) has produced this guide, to help those interested in testing biochar in soil to design and run sound experiments, the results of which can be used to draw strong conclusions and can be disseminated to a wide audience.

As you progress through the various stages of planning, we encourage you to share your thoughts with others and ask questions when there are concepts that you do not understand. One critical design step that you should seek feedback on is the layout of your experiment. Once you have designed a layout you find appropriate and feasible, ask someone who has experience with this kind of experimentation, including data analysis, to look over your work. Specifically, you must ensure not to fall victim of "pseudo-replication". This concept is explained under Step 2, and can be difficult to notice. Pseudo-replication is the same as no replication, and having no replication means it is impossible to carry out any valid statistical analyses on the data. Considering the work that setting up a field trial involves, ensuring a sound layout at the design phase is crucial. A well-planned and sound experiment can provide data over many years.

Before you get started

Before starting to plan the details of your trial, there are several questions that you should address:

- To whom do you want to demonstrate the effects of biochar application to soil? This will dictate
 the setting in which you carry out the trials, for example with large-scale farmers, plot tenders
 in community gardens, coffee growers who are members of a cooperative, etc.
- What resources do you have to work with, both financial and human? This will dictate the size of the trials you can set up, and the kind of data you can collect from them.
- What impacts of biochar do you plan to analyze? If you are looking for yield improvements through better plant nutrition, you should chose a crop that is relevant to your setting while having high fertility demands, and also try to add biochar to the poorest soil that you have: logically that is where it is likely to have the greatest impact. If you are interested in retaining moisture for crops, you need to work in an area and/or with a soil type where moisture is a limitation for crop production.
- How long will your trial(s) last? Keep in mind that the ability of many biochars to retain nutrients develops over time (Cheng 2006, 2008), so it is possible that you will not see differences in the first cropping season after application. Similarly, a single biochar application

has been observed to provide benefits for crop nutrition for several years after an initial "neutral" year.

Once you have a good idea of the kind of trial you want to set up, you are ready to start planning in more depth. The following steps will guide you through a method that aims to ensure reliable, repeatable data are obtained, while making it possible to perform statistical analyses on the data and present them in a convincing way.

STEP 1: Generating hypotheses

This step might seem superfluous, but it is important because defining specific hypotheses to test will help you make sure you collect all the data you need to make your point(s).

Here are some examples of hypotheses for a biochar trial:

Example 1: (i) The addition of 5% biochar to potting medium causes an increase in oil palm seedling size at the normal time of field transplanting, and (ii) after field transplanting, palm survival is improved.

Example 2: (i) The addition of biochar residues from an improved household cooking stove to the home garden area of the household improves the yield of horticultural crops and (ii) the household spends less money for purchasing vegetables from the market OR the vegetable intake in the household's diet is greater.

Example 3: The addition of 20 t/ha biochar to marginal fields on commercial corn growing farms will allow farmers to apply less fertilizer on these fields.

Example 4: The addition of 20 t/ha biochar to marginal fields on commercial corn growing farms will result in a measurable carbon sequestration of 15 t/ha, as measured 10 years after application.

Once you have formed your hypotheses, you can start thinking about how to test them.

Step 2: Planning and designing the trial

What materials will you need? Examples of questions that may need resolving include:

1. What biochar material will you use?

How much do you need? How much will obtaining and shipping the biochar cost? Biochars can be made from a wide variety of biomass materials, and under a wide range of pyrolysis conditions. For these reasons, the resulting biochars can have a range of characteristics, from the beneficial

to the harmful. For example, chars high in volatile organic compounds or salts can harm or kill plants. To test for potential toxicity, it is important to carry out a simple germination test or a worm avoidance test (See Box 1). Also, when making biochar from feedstocks with high silica material such as rice husks, it is important to keep the temperature of pyrolysis low (< 550 °C) to reduce the likelihood of forming crystalline silica. Repeated inhalation of crystalline silica is associated with silicosis, a serious health concern. The IBI strongly recommends obtaining qualified engineering expertise before attempting to build or use any kind of unit to produce biochar. See Boxes 3 and 4 for more information on working with biochar.

Some biochar materials, for example those made from manures and bones are mainly composed of ashes (so-called "high mineral ash biochars"), and thus can supply considerable amounts of nutrients to crops. Keep in mind that this fertilizer effect will likely be immediate and short-lived, just as is the case with synthetic fertilizers. Conversely, the carbon content of high mineral ash biochars is low (e.g. < 10%), and thus longer-term nutrient retention functions will be less for a given amount of material. Also, the potential for soil carbon sequestration might be limited by the low carbon content. Gather as much information as possible on the biochar you will use. Some suppliers inoculate their product with microorganisms or add nutrients to it. Ask if the product has been tested in soil, and if any results are available.

2. Where will you conduct the trials?

Will you work at several locations? How much space will you need at each location? For how long do you need owners/managers of the trial areas to commit to allocating the land to the trials? How secure will the plots be? Do you need to provide special protection against pests or animals at some or all trial locations?

While conducting trials in several locations (e.g. several farms in a watershed or agricultural area, or in contrasting areas) provides a stronger dataset, large projects are more difficult to manage than small ones, and require more resources for carrying out the trials, collecting data and samples, analyzing samples, etc.

3. Who will be in charge of carrying out the trials?

Will you ask farmers to take care of the plots as they usually do? Will you be working on an experimental station where staff are available to manage trials to your specifications? Will you need to hire people? Are the people available to manage the experiment sufficiently qualified to carry out the measurements you need in a consistent and accurate way?

4. What will you measure?

Crop yield? Crop height? Crop quality? Soil fertility? Using a do-it-yourself kit? Sending samples to a lab for analysis? Soil moisture status? Will you need to assess the amount of biochar that is in the soil at different points in time?

The best results on soil fertility and crop nutritional status are obtained from specialized laboratories, and are usually quite costly. This should be considered when deciding on the

number of trial sites, treatments, and replicates that will be used. At the moment, assessing the amount of biochar in soil with acceptable accuracy is expensive.

BASIC CONCEPTS FOR DESIGNING A FIELD LAYOUT FOR THE TRIAL

Concept #1: Control plots

Control (or check) plots are absolutely necessary in any experiment. Without them, no conclusions can be drawn about the effects of anything new that you tried out in the experiment. Control plots set a baseline against which other treatments are compared. Often control plots will be managed according to standard practices, and this "business as usual" situation is compared to adding biochar, for example.

To keep things simple and minimize the number of treatments, plots required, samples to analyze, etc, it is possible to compare only two treatments: control and biochar-amended. In this case the ONLY difference between the two treatments will be the presence or absence of biochar. If more than one factor is different, e.g. a mixture of biochar and compost was used in the "amended" but not in the "control" plots, you will most likely not be able to tell what was responsible for any yield difference: the biochar or the compost or a synergy between the two?

Having said this, larger experiments that address broader questions can also be set up, but care must always be taken to include a valid, "baseline" control. To continue with the example above, a larger experiment including the following treatments would allow you to test the value of several soil amendments:

| Treatment | Fertilization | Amendment 1 | Amendment 2 |
|-----------|---------------|-------------------------------------|--------------------------------|
| 1 | Standard NPK | | |
| 2 | Standard NPK | 10 tons of carbon/ ha as biochar | |
| 3 | Standard NPK | 10 tons of carbon/ ha as compost | |
| 4 | Standard NPK | 5 tons of carbon/ha as biochar | 5 tons of carbon/ha as compost |

Here the effect of compost can be compared to the effect of biochar and to the effect of a mixture of the two (treatment # 2 vs. # 3 vs. #4), and all can be compared to not adding any organic amendment (#1). If "business as usual" is illustrated by #3, #1 could be dropped.

Box 1: Quick tests to determine whether a biochar material contains compounds that are potentially harmful to plants

Biochar can be made from a wide variety of feedstocks and under a wide variety of conditions, yielding a wide variety of potential product characteristics. Not all biochars are suitable for soil application as supplied. Rapid tests such as those described below can be used to assess the presence of toxic compounds in biochar.

Germination test

Methodologies for germination tests are widely available. Basically, the goal is to determine whether adding biochar to soil has an effect on seed germination. It is assumed that a negative effect indicates the presence of undesirable compounds in the biochar material. Lettuce (*Lactuca* sativa L.) is the most widely recommended species to use, due to its sensitivity (US EPA 1994). Other species that could be used include radish (*Raphanus* L.) and clover (*Trifolium* L.). Here are recommended steps:

If possible, use soil from the experimental location where the field trial will take place. If soil from the trial area is not available, use another kind of soil. Remember that the same soil must be used in both the container with biochar and the one without.

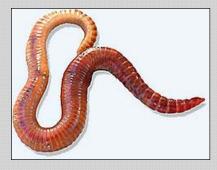
- 1. Obtain two containers with a dish-like shape. These can be plastic lids with a relatively high side, plastic or ceramic plates, etc.
- 2. In one container, place a given amount of soil (measure it out with a cup or other tool).
- 3. In the other, place the same amount of the same soil mixed with your biochar material. The rate of mixing of the biochar can be calculated to simulate the rate you intend on applying in the field, or you can use a ½ and ½ mixture if you don't plan to apply biochar uniformly to soil (but rather in planting holes, for example). In any case, you will have the same volume of soil or soil/biochar mixture in each container.
- 4. Spread the same number of seeds on the surface of the soil in each container. You must use many seeds (20 or more per container), since many seeds might not germinate under any circumstance, and to make sure you get a representative sample.
- 5. Place the containers in a location where good conditions for seed germination occur: normal room temperature is most important. Moisten the soil in each container and make sure it doesn't dry out. Placing a clear plastic bag around the containers helps to prevent drying out.
- 6. Check the containers daily for germination. Once significant germination is observed, count the number of seeds that germinated in each container. Don't wait too long to do this, as seedlings might become entangled and will be harder to count.
- 7. Compare the number of germinated seedlings in the containers with and without biochar, to see if there are differences. You might want to redo the test to convince yourself of the result. Having several replicates of each treatment (with and without biochar) and arranging them randomly while you wait for germination would be even better. See *Concept #2 Replication* for a guide on how to design replications.

Worm avoidance test

This is a more complex test, since it requires live worms to complete. However, it may be more sensitive than a germination test with plant seeds. A common type of worm used for this test is the white worm (*Enchytraeus albidus*). It is widely used as a live aquarium fish food and can be bought where aquarium supplies are sold, or on the internet. Alternatively, worm species *Eisenia fetida* and *Eisenia andrei*, commonly known as redworms, brandling worms, "tiger worms" and red wiggler can be used. Both species are used for vermicomposting and can be obtained from various suppliers.

Here are steps to follow for the test:

- 1. Obtain a flat container as described above. A round container would be best in this case. A diameter around 10 cm is ideal.
- 2. Cut a piece of cardboard or plastic sheeting, so that it will fit along the diameter of your container and to the bottom to split it in half. This will be used to physically separate the soil and soil/biochar mixture during test preparation and when looking for results.
- 3. Place the separator in the container. Using a pen or marker, mark the position of your separator on the edge of the container so you can insert it again at the same place later. In one half, place soil only, and in the other, the soil/biochar mixture. See instructions in #1 and #4 above. Again, use the same amount of soil on each side of the separator. The soil on both sides should be equally moist but not saturated. Beware of watering the soil after removing the separator, as mixing of the two sides might occur.
- 4. Remove the separator, and place 10 worms along the line where the separator was.
- 5. Place the container in an area where normal room temperature is maintained. To avoid drying, you may cover the container with a vented lid or plastic bag with holes in it.
- 6. After 48 hours, place the separator in the same position as before. Thoroughly observe the soil and count the number of worms on each side of the separator. If the worms have avoided the side of the container where biochar was applied, then the biochar should not be applied to soil without further investigation. Again, repeating the test more than once and/or using several replicates will give more conclusive results.







White worms

Note: A standardized methodology for this test is available from the International Organization for Standardization (ISO 17512-1:2008), and can be downloaded from the internet for a fee.

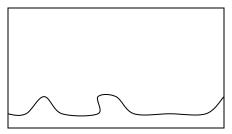
Seeding, weed and pest control must be the same for all treatments. Here, Treatment 1 is the control for testing the effect of biochar and compost. Ideally, to make the comparison as fair as possible, one should modify the amount of inorganic NPK according to the amounts of *available* forms (not total) of these nutrients supplied by the compost and the biochar. In other words, the same amount of available N, P and K should be applied to all treatments. This requires having the biochar and any other amendments analyzed before using them, if information is not supplied with the product.

This experiment has become much larger, in terms of the amount of biochar and experimental land needed, and in terms of the number of measurements and samples it will generate. However, it allows you to assess the effects of two soil amendments as well as the effect of the mixture of the two.

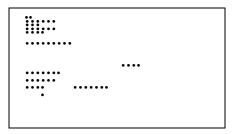
Once you know which treatments you will be experimenting with, you need to decide on a layout for the experiment, be it in the field, nursery, greenhouse or laboratory. Randomized designs with appropriate replication are essential for valid experiments.

Concept #2: Replication

Consider the following two very basic examples of locations for a field trial shown in these hypothetical maps:



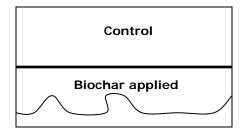
The bottom part of this field gets very wet when it rains and water takes several days to drain

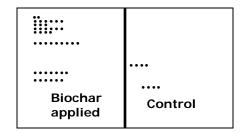


Here, dots represent weed seeds in the soil, thus in this area weed pressure is not uniform. You cannot know this unless you have worked on the plot before and noticed this.

These locations are not uniform with respect to factors that impact crop growth. Furthermore, differences are not always obviously visible or present at any given time. Other examples of experimental areas that are not uniform include shaded zones in a greenhouse, upwind areas in a nursery, etc.

What will happen if you set up field trials in the following way?



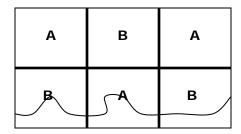


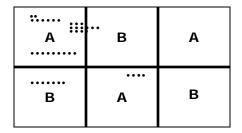
This setup does not provide an unbiased comparison of the control and biochar treatments, because factors other than those we know of and account for (e.g. poor drainage and greater weed pressure) affect one of the treatments more than the other. If you are not aware of these confounding factors (for example if you were not involved with field operations), you might assume biochar actually impacted yields negatively, when in fact other factors are to blame.

To address this problem, you can split the area into several smaller **experimental units**, each of which will contain a **replicate** of a treatment:

A=Control

B= Biochar applied





You can see that the confounding factors now affect plots of both treatments. Thus, even if there are confounding factors of which you are not aware, splitting an area up into many experimental units minimizes the effect of these factors, in terms of treatment comparisons.

In the example above, there are 3 replicates of each treatment. This is the minimum required to conduct statistical analyses of the data, and draw conclusions that will be accepted by the scientific community. More replicates are better because they give more power to detect small but real variations between treatments, but 3-4 replicates are often used to keep the cost and time needed to run the experiment under control. See the important notice on how to avoid pseudo-replication in Box 2.



Non-uniform field plot: here, corn growth is not uniform because the previously forested area was prepared for cropping by slash-and-burn, and the burn was patchy leading to a patchy distribution of ashes. Ashes were the sole fertility amendment in this case.

Photo: J. Major

Replicates will take different forms depending on the setting for the experiment: a replicate can be a potted plant, a seedling tray, a Petri dish, etc.

Once you have decided how many treatments and replicates you will incorporate into your experiment, you can calculate how many experimental units you will need. For example, a simple experiment with 2 treatments and 3 replicates will require 2*3=6 experimental units. How large will the trial be? Multiplying the size of each unit by the number of units will tell you how large your experimental area must be.

The size of experimental units is important as it impacts the validity of the measurements taken, and dictates how much space and inputs you will need. Unit size depends on the type of plant that is used. A 4 by 5 meter experimental unit will contain several hundred maize plants, but maybe only one fruit tree, for example. Thought must be given at this point to the appropriate size to use, in light of the intended sampling techniques that will be implemented, as discussed below in Step 3.

Replication can also be implemented at the landscape scale, where single fields on single farms are used as replicates. Since variability at this scale is presumably much greater than at the scale of a single field, replication must be increased significantly, for example to 10-20 replicates per treatment, depending on variability. This allows conclusions to be drawn which are valid for a much larger geographical area.

Concept #3: Randomization and commonly used layouts

Ideally, treatments should be assigned to experimental units randomly, i.e. avoiding conscious or unconscious bias on the part of the person who decides what goes where. For example, consider the following layout for a uniformly lit greenhouse bench:

| 1 | 2 | 3 |
|---|---|---|
| 4 | 5 | 6 |
| 7 | 8 | 9 |

The bench was divided in as many experimental units as are necessary for the experiment. There are 3 treatments: A, B and C, and 3 replicates for a total of 9 experimental units. In order to assign treatments to units randomly, you can do the following:

In a computer spreadsheet, enter the column as shown:

| - 4 | |
|-----|---|
| | Α |
| | А |
| | Α |
| | В |
| | В |
| | В |
| | С |
| | С |
| | С |

Greenhouse bench layout

These are your three treatments replicated three times. In the column next to it, enter the following formula (for Microsoft Excel): = RAND().

The software will generate a random number between 0 and 1 which will be displayed in the cell. Copy this formula into the cells for the rest of the treatments.

Keep in mind that any time you do anything to the spreadsheet, the random values in the cells change. Once you have your series of random numbers, you can "freeze" them by copying and pasting "values only" (Edit>Paste special>Values) in the same cells.

Then, you can rank the random numbers and their corresponding treatment (select both columns and then chose Data>Sort). Assign each treatment to a number from 1 to 9. Then placing this treatment list into the layout we get:

| Α | 0.444973 |
|---|----------|
| Α | 0.965135 |
| Α | 0.798757 |
| В | 0.40432 |
| В | 0.730048 |
| В | 0.872744 |
| С | 0.183682 |
| С | 0.77423 |
| С | 0.383749 |
| С | 0.183682 |
| С | 0.383749 |
| В | 0.40432 |
| А | 0.444973 |
| В | 0.730048 |
| С | 0.77423 |

0.798757

0.872744

0.965135

| 1 | 2 | 3 |
|-------|-------|-------|
| trt C | trt C | trt B |
| 4 | 5 | 6 |
| trt A | trt B | trt C |
| 7 | 8 | 9 |
| trt A | trt B | trt A |

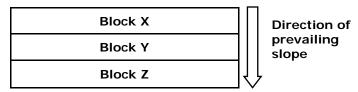
Greenhouse bench layout as Completely Randomized Design (CRD)

This is called a "Completely Randomized Design", or CRD. It is appropriate for experimental areas which are uniform to the best of your knowledge, such as in more controlled settings like a uniformly lit greenhouse.

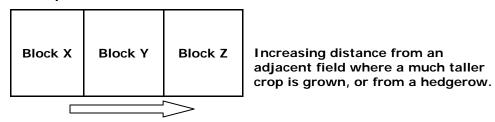
Field conditions are potentially a lot more variable than inside controlled areas. A practice that is very common and appropriate for field experimentation is to choose an area of the required size, that is as uniform as possible, and first divide it into blocks. Blocks are sections that will contain one replicate of each treatment. They are usually designed to be placed perpendicular to the most important know gradient present in the field, if applicable.

Here are some examples:

Example #1:

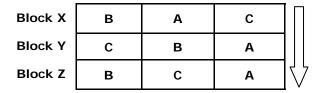


Example #2:



Each block is then divided in as many units as there are treatments, and treatments are assigned randomly as directed above except the procedure is repeated for each block.

Example #1:



Note that each treatment appears once in the "upslope" block, once in the middle and once in the "downslope" block.

Example #2:

| Block X | Block Y | Block Z |
|---------|---------|---------------|
| Α | В | С |
| В | С | Α |
| С | Α | В |
| | | \rightarrow |

Note that each treatment appears once in the block near the adjacent field, once in the middle and once away from the adjacent field.

These two examples represent "Randomized Complete Block Designs", or RCBDs.



An RCBD (Randomized Complete Block Design) with four different biochar application rates as treatments. Notice the three easily identified replicates of the high biochar application rate.

Photo: J. Major.

Step 3: Planning measurements and sampling activities

Depending on the hypotheses to be tested, a number of measurements and/or samples may need to be taken.

These should be determined as far in advance as possible to allow proper planning. Indeed while some samples can be relatively easy and cheap to collect (e.g. plant tissue samples), having them analyzed professionally is usually expensive. Calculate how many samples of each type you would have, and the cost of analysis, in order to decide whether to collect the samples in the first place. Although a wide range of measurements could be taken from experimental plots, here we will outline ideas to be taken into consideration for basic components of most trials: yield measurements and soil sampling.

YIELD MEASUREMENTS

Typically, yield is measured on several plants, from one or many areas inside each experimental unit. This is not to be confused with replication. Several plants are analyzed to account for variability between single plants. Data for all plants inside an experimental unit will be averaged into one value for the experimental unit. In the case where an experimental unit contains a single plant, e.g. in a nursery trial with trees or palms, replication should be increased to more than 3 in order to facilitate the detection of differences between treatments.

When working with field crops or forages where several hundred plants grow in each experimental unit, one can measure yield from a specific area (e.g. 2—one meter square quadrats (frames) for a forage legume, or from a given row length (e.g. 4 times one linear meter of corn row). As you might guess, the more plants are included the better, but smaller sets of plants require less work. Make sure you also collect data required to scale up your yield data (e.g. the number of plants in each linear meter, number of plants per

Box 2: A note on pseudo-replication

Replication is crucial in well-designed experiments. It allows one to ensure that results are due to the factors being tested and not to other random factors such as a treatment falling on a bad spot in the field, or being the victim of an animal that only visits one part of a screenhouse. However, experimenters who intend to design a replicated trial may fall into the trap of pseudo-replication, which is equivalent to no replication.

Here is an explanation of what pseudo-replication is:

Sometimes it may seem that an experiment can be made a lot simpler by not applying treatments in distinct experimental units, in a patchy pattern as shown in examples above.

For example, you can decide to apply biochar in a single swath in the field, or to an entire row of trees in an orchard, with an untreated row or swath beside it serving as a control. This indeed makes application easier than having to create several squares with biochar, interspersed with untreated squares. One could then plan on having "replicates" that are several trees in the orchard row, or several quadrats or linear meters in the corn swath.

The problem with this layout is illustrated with the non-uniform fields under Concept #2 above. Valid statistical analyses cannot be carried out using data from pseudo-replicated experiments. For an experiment to contain valid replication, experimental units must be independent from each other. When all (pseudo) replicates are taken from one single, continuous area where the treatment was applied, they are not independent and there is no real replication.

hectare in the field, etc.)

The specific location of quadrats or linear meters can be decided upon systematically (e.g. 2 m² in the center of each plot) or randomly (e.g. by tossing the quadrat frame inside the plot). However, the edges of the experimental unit should be avoided. The edges are more likely to have been affected by nearby treatments or conditions outside the experiment. Depending on the crop grown, harvested biomass may need to be weighed fresh and/or after drying. Are you interested only in marketable parts of the plant or in the biomass of the entire plant?

The discussion above implies hand harvesting. Indeed if small plots are used, it will be very difficult to use machinery to accurately measure yield. Larger plots for machinery harvesting can be used if sufficient amounts of biochar are available to generate a replicated design with plots large enough to get acceptably accurate data from yield measuring devices on machinery. Plots may need to be very large to accommodate full sized farm machinery and allow individual experimental plots to be assessed separately.

SOIL SAMPLING

Soil sampling may sound like an easy and simple undertaking; however, doing it well (i.e. representatively) requires taking several factors into consideration.

1. When to sample?

If the trial is to last for more than one growing season, yearly soil sampling should take place as much as possible at the same time each year, since nutrient availability changes with cropping and other yearly cycles. Sampling just after harvesting and/or just before planting is easiest practically. Soil that is very wet or very dry can be extremely hard to sample, so avoid periods when such conditions are likely to prevail.

2. Where to sample?

Soil conditions can vary within short distances. You should always dig samples from more than one location in each plot. For example, inside a 4*5 m corn plot, soil can be taken from 3-5 locations. As is the case for measuring yield, plot edges should be avoided. Either a random or regular pattern (e.g. 4 corners inside of edges and the center) can be applied, with the same pattern used inside each experimental unit. Samples from each location can then be combined as described below.

In the case where there are defined crop rows, you should take this into consideration when sampling, especially if fertilizer is banded along these rows. For example, 3 of the sampling locations inside all plots could be on crop rows, and 2 at mid-points between rows.

3. How to sample?

Several tools can be used to sample soil. A variety of augers and corers are available for this specific task, but a trowel or shovel can also be used. The important point is to be consistent in the way any tool is used. For example, if using a shovel, the same amount of soil from a uniform area and depth should be taken from each sampling location. This is important because a steep gradient may be present, with depth, for the soil factors you intend to get information on. Some soil-sampling augers must be used with care, as the very top of the sample might fall off and not be included in the sample. This can happen with Dutch augers, which are otherwise well suited to sampling clays.

Surface soil (e.g. to 10-20 cm) is often used because it is within this increment that most crops obtain nutrients. You should determine the depth at which you will sample soil and be consistent.

For analysis, using one composite sample per experimental unit is appropriate. To obtain a composite sample, place all subsamples from the various sampling locations inside each experimental unit inside a bucket and mix well by hand, breaking large clumps. Then a composite sample of, say 300 g can be taken into a labeled plastic bag, and the rest of the soil discarded. This ensures that you get a homogeneous sample that represents the entire

experimental unit. Labeling bags before setting out to sample is a good idea to avoid mislabeling.

Since biochar can be expected to have a lower density than soil, if you are interested in analyzing changes in carbon stocks after biochar application you may consider sampling the soil's bulk density. This will allow you to accurately report soil carbon stocks. Bulk density is usually measured by taking soil in undisturbed cores (aluminum cores are specifically made for this purpose), and then drying the soil completely. After determining the mass of the dry soil (minus the core), this value is divided by the volume of soil to give bulk density.

4. What to do with the samples?

While some soil analyses require moist soil (e.g. inorganic nitrogen, soil biota), most standard analyses are done using air-dried soil. Since keeping soil moist inside bags can favor the growth of molds and affect natural processes related to soil fertility, soil should be set out to dry as soon as possible after sampling. Dry soil is also easier and cleaner to manipulate and ship to the laboratory. In a covered area (to avoid contamination for example from dust) with ample table space, spread each sample out over pieces of plastic or paper, and leave it for several days until it looks and feels dry. Make sure you don't lose track of sample labels while drying them. It's a good idea to keep some archived soil samples in storage, to have the ability to re-run analyses or run different ones later.

5. Optional but important: sampling biochar in soil

Very valuable data could be obtained from biochar particles picked from soil, after having spent some time in the field. Ideally, several hundred grams of biochar pieces could be physically separated from soil on a yearly basis. This might not be possible with very fine biochar materials. If you do gather such samples, air dry and store them in a dry place inside a glass jar, and contact IBI for information on where to send the sample(s).

Step 4: Carrying out the trial

Make sure you keep a representative sample of your biochar material for analysis.

If you use biochar from different sources, make sure you blend all materials well and apply the same product to all plots. To obtain a representative sample, sample from many locations in a homogeneous biochar pile, or from several barrels or bags of homogeneous material. All of your "subsamples" can be pooled and constitute a single sample of the material you applied.

All experimental units should be managed identically, except for the factors that constitute the treatments. For example, unless you are testing these factors, tillage, weed and pest and disease control should be uniform on all units. It is important for those who carry out field operations to

Box 3: A note on handling and applying biochar

Biochar is a very light and brittle material of low density, and even if it is not fine grained as some biochars are, it usually contains a fraction of fine powder.

Care must be taken when transporting biochar because of the risk of accidental ignition with some biochar materials. This is why commercial products are often moistened before shipping (thereby increasing the product's weight).

Significant amounts of material can blow away while measuring, transporting, applying and incorporating biochar. It is important to develop local systems of producing, storing and transporting biochar. Additionally, dust problems can be controlled by adding small amounts of water to the biochar, mixing with clay slurry, pelleting, agglomerating or prilling with a binder. Incorporating biochar well into soil will minimize surface runoff with water after heavy rainfall events, and/or wind erosion.





Transporting an unprocessed, fine biochar material to the field, applying and incorporating it in St-François-Xavier-de-Brompton, Québec. Both images show biochar blowing off. Photos courtesy of Blue Leaf, Inc., www.blue-leaf.ca.

understand this, and not identifying treatments by name in the field (with signs, for example) might help reduce bias when field operations are carried out. For example, if you have an interest in certain treatments giving better results, or know your boss does, you might be tempted to apply more pesticides or fertilizer over the plots that you would like to see perform better. This might happen unconsciously.

Communication is critical when trials are carried out at several locations and managed by different people, for example farmers. If you need them to manage the trial according to your specifications, you must make sure they agree to this and understand what you need. Be prepared for changes to be made to your plans: farmers deal with complex situations on a daily basis and might make decisions that you had not anticipated.

Box 4: Biochar application to soil

(adapted from Blackwell et al. 2009)

Biochar can be applied along with other amendments like compost, manures or crop residues, and it does not need to be applied when each new crop is established to provide benefits over time. Biochar will likely be applied for agricultural profitability and/or carbon sequestration. Thus, applying the material must not increase costs and/or CO_2 emissions beyond acceptable levels.

Several techniques can be considered for biochar application to soil. At all times, controlling erosion by wind and surface runoff with water must be kept in mind. Erosion losses are undesirable because they represent a waste of biochar, and this lost material is not available in the soil for improving its fertility or sequestering carbon on a given land area. For many of these techniques, biochar can be mixed with other soil amendments such as compost, manure, crop residue, etc.

Uniform topsoil mixing

Biochar is broadcast over the entire application area, usually after primary soil preparation (e.g. by hand hoeing or disk tilling). This method can be used before crop establishment.



Uniform topsoil mixing of biochar carried out manually in Honduras. Photo: J. Major

Application can be done using lime spreaders or other spreaders. Biochar can also be applied as a liquid slurry, possibly mixed with liquid manure. After application, incorporation is achieved by hand or with disking or chisel tillage, for example. The most appropriate methods will depend on soil conditions and individual farm capacities. Uniform application could also be considered during the establishment of turf, golf greens, athletic fields, and general landscaping after construction. Thorough biochar mixing and covering with soil is then possible. Uniform biochar topsoil mixing is the incorporation technique most widely used to date.

Application to planting holes

When establishing orchards, tree or palm plantations, application of biochar to individual planting holes is another technique that minimizes erosion losses.

(Continued on page 21)

(Continued from page 20) Box 4: Biochar application to soil



Biochar being banded in a Western Australia wheat field. Photo: P. Blackwell.

Banding

Biochar can be banded at different depths, again by hand or using machinery. Deep banding may facilitate thoroughly covering the biochar with soil, thereby minimizing potential losses after application. This is also an option where crops or trees are already established.

Around trees, a circular band of biochar can be applied, or several holes can be made around the base of the tree and biochar applied to the bottom of these holes.



Compost being top-dressed in an orchard. Biochar could be applied similarly. Photo: M. Collins.

Top-dressing

Also in areas where crops are already established, topdressing is an option where biochar is applied to the soil surface. However, this method has the highest potential for erosion losses. Mixing biochar with other amendments, applying it to flat land with a thick vegetation cover, mulching, etc are all ways of reducing potential losses. Using minimal tillage is also possible in some cases.

Step 5: Having soil samples analyzed

While do-it-yourself kits are available to analyze certain properties of soils (like pH, for example), reliable analysis of soil fertility is done by specialized laboratories.

A soil-testing lab in your region can conduct analyses in ways that are designed for the soils of your region. This is important because several methods are available for analyzing soil properties and yield different (but usually correlated) results. To find a soil testing lab in your region, you can contact cooperative extension services, universities with agronomy departments, gardening stores, etc. Labs often have online order forms and offer analysis packages.

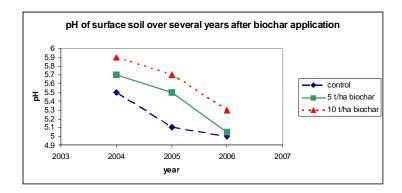
Methods for quantifying biochar in soil specifically are being developed, and currently no routine

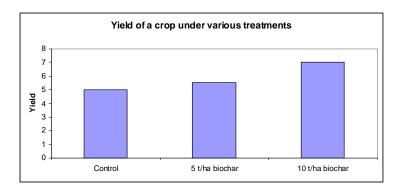
analyses are offered on a commercial basis to do this. Therefore, obtaining these data is not yet available for the public. It is currently easier to analyze the soil for total carbon, and to compare how much total carbon is in biochar-amended soil and control soil that did not receive biochar. The control soil will tell you how much non-biochar carbon is in the soil. You must beware of the analysis you choose to determine total carbon, since biochar, as we know, is very difficult to degrade and not all routine analyses for soil carbon pick it up. The analysis known as organic matter by "loss on ignition" (LOI) is inexpensive, but will not provide data on carbon amounts from biochar. Methods that are known as "wet chemical" (i.e. involving liquid chemicals being added to the soil), such as dichromate oxidation, also do not quantify carbon from biochar. You must request a total carbon analysis, which is carried out by dry combustion in a C/N analyzer. If in doubt, contact the lab and explain what you need.

Step 6: Analyzing and presenting results

Once you have data in hand, it is time to determine what are the results of all your work.

Since you have 3 or more replicates of each treatment, you can calculate average values for yield, available phosphorus, pH, etc., in each treatment. A good way of presenting data is graphically. Figures allow others to quickly and visually understand results. The following are examples of graphical ways of presenting data:





Both these figures were generated using Excel; other spreadsheet programs can be used to generate similar graphs.

These figures show differences between treatments that are sometimes small. With data from a well-designed experiment, it is possible to tell whether these differences are real or whether they must be attributed to random variability in the field, in the way measurements were made, etc. Statistical analysis is used to assess the significance of differences between treatments, and these require lengthy calculations or the use of specialized software. If you are not familiar with these, you should seek the help of someone who is. In many cases, outside the scientific world, such analyses are not necessary to use and disseminate results.

Here we will explain a tool that will allow you to illustrate, using figures, the amount of variability in your data. Variability refers to the "spread" in the values you used to calculate an average. Consider the following example:

| Replicate | yield |
|-----------|-------|
| 1 | 5 |
| 2 | 10 |
| 3 | 15 |
| average | 10 |

| Replicate | yield |
|-----------|-------|
| 1 | 11 |
| 2 | 9 |
| 3 | 10 |
| average | 10 |

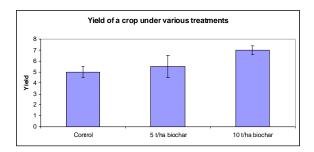
You can see that the data on the left are more variable than the data on the right, even though the average is 10 in both cases. When comparing treatments that are highly variable, the average by itself may give a misleading impression. Consider the following:

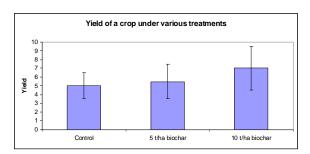
| Treatment | Replicate | yield |
|-----------|-----------|-------|
| Control | 1 | 5 |
| Control | 2 | 10 |
| Control | 3 | 15 |
| | average | 10 |
| Biochar | 1 | 20 |
| Biochar | 2 | 15 |
| Biochar | 3 | 10 |
| | average | 15 |

| Treatment | Replicate | yield |
|-----------|-----------|-------|
| Control | 1 | 11 |
| Control | 2 | 9 |
| Control | 3 | 10 |
| | average | 10 |
| Biochar | 1 | 15 |
| Biochar | 2 | 13 |
| Biochar | 3 | 17 |
| | average | 15 |

While averages are again the same for the same treatment, when looking at the data on the left one could have doubts as to whether biochar is really better than the control. Is the value for that first replicate in the biochar treatment really accurate? If not, then even though the average for the biochar treatment would still be greater than for the control, all of the data would still fall in the range of the data obtained in the control. The example on the right shows data that is much more convincing. How can you show this graphically?

Adding error bars to figures gives an idea of the amount of variability in the data. The longer the bar, the more variable, or spread out, is the data. Basically, the error bar gives an idea of the range in which the actual data bar could lie. The wider the range, the more probable it is that one data bar overlaps with another, indicating that differences between the two are not that strong. When comparing two treatments with overlapping error bars, one can doubt the significance of the difference between the treatments. These bars commonly represent the standard error of the data. Consider the following examples:





It is clear that in the left hand figure, we can be confident that 10 t/ha of biochar caused an increase in yield over the control, while we are less confident about the 5 t/ha application rate. In the right hand figure, biochar application seems to give very variable results and it is difficult to draw strong conclusions from the data.

How can you generate error bars on graphs?

In Microsoft Excel, follow this procedure to calculate a standard error for each treatment average:

In the cell where you want to calculate the standard error, enter the following formula: =STDEV (select the range of data you used to calculate the average) / SQRT (number of data points you have, usually 3). Once you have standard error values for all treatments, generate a graph with the averages. On the graph, right click on a dot or bar, and select "Format data series".

Then choose the tab "Y error bars". You can then select the style of bar you prefer. Under "error amount", choose "custom". Using the selector tool to the right of each of the fields provided, select the standard errors corresponding to the set of averages you plotted.

Enter the same range for the positive and negative bar. This will be easiest if the standard errors are in a column beside the averages.

Step 7: Disseminating results

A lot of very interesting trials are never taken through the last and very important step of dissemination. Sharing your results with others will help them make decisions about using biochar, and perhaps expand on your results to increase everyone's level of knowledge.

In many cases, field trials were undertaken to convince people of something, and so revealing the results of the trial is obviously important. Field trials can be a lot of work and keeping results to yourself or only circulating them within your organization or to a specific group of interest fails to bring the work to its full potential.

Depending on what your objectives were when undertaking the trial, there are usually several outlets that can be used to share your results. Some of these include posting a report on the internet, writing an article for a newsletter aimed at your target audience, presenting your results at a meeting, etc. The IBI has begun a registry for documenting field trials. You are invited to apply to join the IBI Field Trial Registry, where you may input your information in a format that will lead to broad visibility of your results.

The IBI Biochar Trial Registry

To join the IBI Field Trial Registry, contact the IBI Extension Director:

Julie@biochar-international.org

Send an email to IBI Extension Director, Julie Major, at the address above.

Please be aware that by agreeing to join the IBI Field Trial Registry, you agree that all of the data you provide will be open and accessible to the public.

Data forms for the Biochar Trial Registry are included in the appendix to this guide. Soon, IBI will make these available in electronic form. For now, use the paper forms to record your field trial data.

You are welcome to use these data forms for your experiments whether or not you join the IBI Biochar Trial Registry.

For further questions, look for updates on the IBI website: **www.biochar-international.org** or you may contact IBI Extension Director, Julie Major, at julie@biochar-international.org.

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Appendix: Trial Data Sheets

- 1. Basic Project Information
- 2. Char Characterization
- 3. Experimental Design
- 4. Soil Analysis Results

| Registered Biochar Trial - Basic Project Info | | | | | | | | |
|---|---------------------|-----------------|-----|------------------------|------------|--------------------|----|--|
| Name of project adminis | strator | | | | | | | |
| IBI member ID number | | | | | | | | |
| Project ID number | | | | | | | | |
| Project Title | | | | | | | | |
| Type of organization (accommercial, governmen | cademic, it, NGO | | | | | | | |
| mailing address | | | | | | | | |
| phone numbers, skype | | | | | | | | |
| email, web address | | | | | | | | |
| preferred language | | | | | | | | |
| partners or cooperators | | | | | | | | |
| site location (decimal de | grees) | latitude | | <mark>longitude</mark> | | | | |
| project start date dd/mn | n/yy | | | | | | | |
| project end date dd/mm/ | /yy | | | | | | | |
| | | | | soil type data | | | | |
| soil type | | soil text | ure | | s | oil detail | | |
| place X in box next to choice | | place X in bo | | | additional | description of soi | I: | |
| Alfisol/Luvisol | | silt | | | | | | |
| Andisol | | silt loam | | | | | | |
| Andosol | | sandy clay loam | | | | | | |
| Arenosol | | clay loam | | - | | | | |
| Aridisol | | silty clay loam | | - | | | | |
| Cambisol | | sandy clay | | | | | | |
| Entisol | | silty clay | | | | | | |
| Fluvisol | | | | | | | | |
| Gelisol | | | | | | | | |
| Gleysol | | | | | | | | |
| Histosol | | | | | | | | |
| Inceptisol | | | | | | | | |
| Lithosol | | | | | | | | |
| Mollisol | | | | | | | | |
| Nitosol Oxisol/Ferralsol/Latosol | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| Regosol | | | | | | | | |
| Spodosol/Podzol Ultisol/Acrisol | | | | | | | | |

| Biochar Characterization and Analysis | | | | | | | | | |
|--|-------------------------------|----------------|----------|--------|---------|----------------------------------|---------------------|----|--|
| IBI member ID number | | | | | | | | | |
| Project ID number | | | | | | | | | |
| biochar feedstock | | | | | | | | | |
| feedstock supplier | | | | | | | | | |
| feedstock pre-treatment | | | | | | | | | |
| production method | | | | | | | | | |
| peak production temperature (C) | | | | | | | | | |
| amendments/inoculants added to char or other pre- application treatment | | | | | | | | | |
| Describe methods and results of seed germination test | | | | | | | | | |
| Describe methods and results of worm avoidance test | | | | | | | | | |
| | | Bioch | nar Lab | Analy | sis Da | ta | | | |
| name of lab used: | | <u> </u> | <u> </u> | ruidiy | 0.0 00 | | | | |
| Hame of lab used. | | | | | | | | | Date to the state of the state of |
| Characteristic | | Units / values | | | | | | | Brief description of analysis method Required - or data will not be used |
| pH (in H | 20) | | | | | | char/water ratio | рН | |
| | enter m | olarity | | | | | char/solution ratio | рН | |
| pH in KCL or CaCl2 | of sol | | KCI | | CaCl2 | | | | |
| Total C | us | ea | | | | <u>%</u> | | | |
| Total N | | | | | | % | | | |
| C/N | | | | | | ratio | | | |
| H/C | ratio | | | | | | | | |
| O/C | ratio | | | | | | | | |
| Ash | % | | | | | | | | |
| Volatile C | <u>%</u> | | | | | | | | |
| Fixed C | <u>%</u> | | | | | | | | |
| Labile C | % | | | | | | | | |
| Unit options - place X in box next to chosen unit | mg g-1 μg g-1 (ppm) mmolckg-1 | | | | | | value | | |
| Available Ca | | | | | | | | | |
| Available Mg | | | | | | | | | |
| Available P | | | | | | | | | |
| Available K | | | | | | | | | |
| other available element please name: | | | | | | | | | |
| other available element please name: | | | | | | | | | |
| other available element please name: | | | | | | | | | |
| Total Ca | | | | | | | | | |
| Total Mg | | | | | | | | | |
| Total P | | | | | | | | | |
| Total K | | | | | | | | | |
| other total element please name: | | | | | | | | | |
| other total element please name: other total element please name: | | | | | | | | | |
| Surface area | | | | | | m ² g ⁻¹ | | | |
| CEC | | | | | mm | ol _c kg ⁻¹ | | | |
| | | values | | | | <u> </u> | | | |
| Pore volume and size range | vol, cm3g-1 | | Min, nm | | Max, nm | | | | |
| Pore volume and size range | | | Min, nm | | Max, nm | | | | |
| Pore volume and size range | | | Min, nm | | Max, nm | | | | |
| Pore volume and size range | | | Min, nm | | Max, nm | | | | |
| Pore volume and size range | | | Min, nm | | Max, nm | | | | |
| Other data: | | | | | | | | | |
| Other data: | | | | | | | | | |
| Other data: | | | | | | | | | |
| Other data: | | | | | | | | | |
| | • | | | | | | | | • |

| Experimental Design and Results | | | | | | | | | |
|--|------------------------------|---|-----------------------|------------------------|-------------------|--------|--|--|--|
| IBI member ID number | | | | | | | | | |
| Project ID number | | | | | | | | | |
| experimental setting - place X in box underneath | | pots in greenhouse | pots outside | field | Other (please spe | cify) | | | |
| | | | | | | | | | |
| experimental design - place X in box underneath | | Randomized Complete Block Design (RCBD) | No randomiza- tion | Other (please specify) | | | | | |
| | | | | | | | | | |
| number of replications | ; | | | | | | | | |
| | | Applicatio | n | | | | | | |
| biochar application rate -choose units | tons/ha | kg/ha | g/kg soil | | | | | | |
| and fill in number | | | | | | | | | |
| depth of application | cm | | | | | | | | |
| describe method of application and incorporation | | | | | | | | | |
| dates of biochar application (dd/mm/yy) | | | | | | | | | |
| опос от отсетот организм (отпользять уут | type | rate | type | rate | type | rate | | | |
| other amendents | | | 77.5 | | 77. | | | | |
| dates of application (dd/mm/yy) | | | | | | | | | |
| Describe your control treatment | | | | | | | | | |
| | | Irrigation | | | | | | | |
| Irrigation method - place X in box under- | D. C. C. I | | | EL. I | | | | | |
| neath | Rainfed Drip Sprinkler Flood | | | | | | | | |
| describe irrigation details | tails | | | | | | | | |
| | | Planting | | | | | | | |
| crop species planted | | | | | | | | | |
| planting density | units in plants/ha | | | | | | | | |
| | dav | month | year | | | | | | |
| planting date | | | | | | | | | |
| | day | month | year | | | | | | |
| harvesting date | | | | | | | | | |
| | | Results | | | | | | | |
| | # of soil cample resu | Its submitted on sepa- | | | | | | | |
| soil sampling results | | forms: | | | | | | | |
| soil carbon measurements (describe results) | | | | | | | | | |
| greenhouse gas emission measure- ments (describe results) | | | | | | | | | |
| percent yield increase over control | biochar only | other: | | other: | | other: | | | |
| detailed narrative of results | | | | | | | | | |

| Soil Analysis Results | | | | | | | | | | |
|---|--------------------------|----------------------|--------------------------|-----|-----------|-------|---------------------|---------------------|----|---|
| IBI member IE | number | | | | | | | | | |
| Project II | D number | | | | | | | | | |
| sample date (d | dd/mm/yy) | | | | | | | | | |
| sampl | e location | | | | | | | | | |
| name o | f lab used | | | | | | | | | |
| characteristic | : | | Units / secondary values | | | | | | | Brief description of analysis method required - you must provide this information or your data will not be used |
| pH (in H2O) | | | | | | | | char/water ratio | рН | |
| pH in KCL or CaC | CI2 | enter mo solution | larity of n used | KCI | | CaCl2 | | char/solution ratio | рН | |
| | Total C | | | | | | % | | | |
| | Total N | | | | | | % | | | |
| | C/N | | | | | | ratio | | | |
| | H/C | ratio | | | | | | | | |
| | O/C Ash | | | | | | ratio % | | | |
| | Volatile C | | | | | | % | | | |
| | Fixed C | | % | | | | | | | |
| | Labile C | | | ı | | | % | | | |
| Unit options - place next to che | | mg g-1 µg g-1 (ppm) | | | mmolckg-1 | | value | | | |
| Ava | ailable Ca | | | | | | | | | |
| | ailable Mg | | | | | | | | | |
| | vailable P vailable K | | | | | | | | | |
| | | | | | | | | | | |
| other available element please name: | | | | | | | | | | |
| other available element please name: | | | | | | | | | | |
| other available element please name: | | | | | | | | | | |
| | Total Ca | | | | | | | | | |
| | | | | | | | | | | |
| | Total P | | | | | | | | | |
| other total element please name: | Total K | | | | | | | | | |
| other total element please name: | | | | | | | | | | |
| other total element please name: | | | | | | | | _ | | |
| CEC | ı | | | | | mmol | l₀ kg ⁻¹ | | | |
| Other results, please describe | | | | | | | | | | |