Terrestrial Environmental Effects Monitoring

Forest Health Monitoring Program

Procedures Manual

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1.1 Background

Sample labeling is a critical activity in the preservation of sample integrity. Incorrect, incomplete, ambiguous, worn and lost labels create uncertainty regarding the contents of the sample, possibly causing the sample to be discarded, a loss of data and of the financial resources invested in its collection.

1.2 Summary of Labelling Requirements

Labelling procedures are meant to convey all necessary sample information in a permanent and redundant manner, reducing the potential for loss of label information by wearing of an individual label or loss of a label, respectively. A minimum of two labels is to be attached to all samples, using two different methods. The methods are specific to sample type, summarized in the following table and as defined in detail by sample type in the sections that follow.

Required Information	Format	Soil		Needles		Tree Core		Bark	Lichen
		Pit	Plot	Off-Plot	Edge Plot	Off-Plot	Edge Plot		
Sample Date	YYYY-MM-DD	X	X	X	X	X	X	X	X
Site	5-character site name	X	X	X	X	X	X	X	X
Field Personnel	Initials	X	X	X	X	X	X	X	X
Horizon Designation	Soil pit horizon; add suffix "-x" to the horizon designation to identify field duplicate	X							
Soil Plot	Plot number, from 1 to 4; field duplicates labelled from A to D, for plots 1 to 4, respectively		X						
Soil sample location	Sample location, from 1 to 9 (or 1 to 6 in smaller plots)		X						
Depth Increment	One of: "LFH", "0-5cm", "5-15cm", "15-30cm", "30-50cm"		X						
Tree Number	3-character tree label ("010", "X10" or "E10"); field duplicates as "Dxx", where "xx" is the tree number			X	X	X	X	X	
Sample Age Class	One of: "CAG", "Age-1", "Age-2"			X	X				
Lichen species	"*Hypogymnia physoides*"								X

1.3 Soil Samples

Soil samples are to be contained in a plastic zipper storage bag, which placed into a second plastic sealable zipper bag. Both bags are to be labelled, and a paper label placed between the two bags.

Label information for soil samples is defined in the table below. A permanent blue or black marker (e.g., "Sharpie" brand) is to be used to directly label plastic sample bags. Waterproof paper (e.g., "Write in the Rain" brand) and pencil are required to prepare paper labels to insert between sample bags.

Required Label Information for Soil Samples

Required Information	Soil Pit	Soil Plot	Required Format
Sample Date	X	X	Date as YYYY-MM-DD (July 9, 2011 would be recorded as "2011-07-09")
Field Personnel	X	X	The initials of the person(s) collecting the sample. Three initials (including middle initial) are to be used; if initials create ambiguity (e.g., John M. Smith and Jane M. Shaw conducted the sampling), use of a first or last name on the sample label is required to uniquely identify individuals.
Site	X	X	The 5-character sample site designation
Horizon Designation	X		The horizons sampled from the soil pit, abbreviated according to Canadian terminology. The abbreviation used on the label is to exactly match that entered into TEEM Form 10*. Field duplicates are to be indicated by adding the suffix "-x" to the horizon designation. Thus, the field duplicate for the LFH sample would be identified as "LFH-x"
Soil Plot		X	Soil plot number, from 1 to 4 The field duplicate sample is to be labelled from A to D, with "A" representing soil plot 1, "B" representing soil plot 2, "C" representing soil plot 3, and "D" representing soil plot 4, respectively
Soil Subplot		X	Soil subplot number, from 1 to 4. This applies to the field duplicate sample as well
Soil sample location		X	Sample location, from 1 to 9 (or 1 to 6 for smaller plots). This applies to the field duplicate sample as well
Depth Increment		X	One of: LFH 0 to 5 cm 5 to 15 cm 15 to 30 cm 30 to 50 cm (site establishment year only)

* TEEM Form 10 is part of the **SOIL DESCRIPTION PROCEDURE (#7)**

1.4 Jack Pine Needle Samples

Harvested shoots containing needles of a specific age class are to be collected into a plastic zipper bag in the field. At the end of the field day, samples are to be transferred to a brown paper bag for storage or shipping to the laboratory.

Label information for needle samples is defined in the table below. A permanent blue or black marker (e.g., “Sharpie” brand) is to be used to directly label plastic or paper sample bags, and waterproof paper (e.g., “Write in the Rain” brand) and pencil are required to prepare paper labels to insert into the sample bags.

Required Label Information for Jack Pine Needle Samples

Required Information	Interior Site	Edge Site	Required Format
Sample Date	X	X	Date as YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
Field Personnel	X	X	The initials of the person(s) collecting the sample. Three initials (including middle name) should be used; if initials create ambiguity (e.g., John M. Smith and Jane M. Shaw conducted the sampling), use of a first or last name on the sample label is required to uniquely identify individuals.
Site	X	X	The 5-character sample site designation
Tree Number	X	X	The 3-character tree label (e.g., “X06” for off-plot trees, “E06” for edge plot trees) The field duplicate sample is to be labelled in the format “Dxx”, where “D” stands for “field duplicate”, and “xx” represents the tree number from which the needles were taken (e.g., “D06”)
Sample Age Class	X	X	One of: CAG (Current Annual Growth) 1-Year-Old 2-Year-Old

1.5 Tree Core Samples

Tree cores are to be placed into plastic straws, the ends stapled closed, and a label attached to one end of the straw. A masking tape label is to be prepared by wrapping a length of tape around one end of the straw, and sticking one end of the tape to the other create a writing space of about 3 cm length. A permanent blue or black fine-point marker (e.g., “Sharpie” brand) is to be used to write the sample information on the tape. Label information for tree core samples is defined in the table below. This is the only sample type where a single label is permitted.

Required Information	Interior Site	Edge Site	Required Format
Sample Date	X	X	Date as YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
Field Personnel	X	X	The initials of the person collecting the core. Three initials (including middle name) should be used; if initials create ambiguity (e.g., John M. Smith and Jane M. Shaw conducted the sampling), use of a first or last name on the sample label is required to uniquely identify individuals
Site	X	X	The 5-character sample site designation
Tree No.	X	X	The 3-character off-plot tree number, in the form “X10”

1.6 Bark Samples

Bark samples are to be collected into a plastic zipper storage bag, which placed into a second plastic sealable zipper bag. Both bags are to be labelled, and a paper label placed between the two bags.

Label information for bark samples is defined in the table below. A permanent blue or black marker (e.g., “Sharpie” brand) is to be used to directly label plastic sample bags, and waterproof paper (e.g., “Write in the Rain” brand) and pencil are required to prepare paper labels to insert between sample bags.

Required Label Information for Bark Samples

Required Information	Required Format
Sample Date	Date as YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
Field Personnel	The initials of the person collecting the sample. Three initials (including middle name) should be used; if initials create ambiguity (e.g., John M. Smith and Jane M. Shaw conducted the sampling), use of a first or last name on the sample label is required to uniquely identify individuals
Site	The 5-character sample site designation
Tree Number	The 3-character off-plot tree label (e.g., “X09”) The field duplicate is to be labelled with a “D” in place of the “X” in the tree number (e.g., the duplicate bark sample from tree X09 is to be labelled “D09”; the bark from tree E04 is to be labelled “D04”)

1.7 Lichen Samples

Lichen samples are to be collected into a plastic zipper storage bag, which placed into a second plastic sealable zipper bag. Both bags are to be labelled, and a paper label placed between the two bags. A permanent blue or black marker (e.g., “Sharpie” brand) is to be used to directly label plastic or paper sample bags, and waterproof paper (e.g., “Write in the Rain” brand) and pencil are required to prepare paper labels to insert into the sample bags. Label information for lichen samples is defined in the table below.

Required Label Information for Lichen Samples

Required Information	Required Format
Sample Date	Date as YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
Field Personnel	The initials of the person collecting the sample. Three initials (including middle name) should be used; if initials create ambiguity (e.g., John M. Smith and Jane M. Shaw conducted the sampling), use of a first or last name on the sample label is required to uniquely identify individuals
Site	The 5-character site designation
Lichen species	*Hypogymnia physodes*
Replicate number	1, 2 or 3. The field duplicate is to be identified with an “x” following the replicate number (e.g., “2x”)

1.8 Labelling Requirements when Transferring Samples

The information on a sample label must be completely transferred to a new container when transferring the sample to that container. If replacing a container with the same type of container, the label(s) on and/or in the new container must be of the same type and configuration as those associated with the old.

1.9 Label Replacement

Each time a sample is handled, the labels on the sample are to be examined. Damaged labels are to be replaced using the same materials as were used in the original label, and the labels must contain the same information as was contained on the original label, in its entirety.

Ultimately the label information created and affixed to a sample in the field should be no different in content than that affixed to a sample when it is placed into the TEEM archive.

PROCEDURE #2
SAMPLE STORAGE & SHIPPING

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2.1 Background

The samples acquired in the TEEM Forest Health Monitoring Program are biological (e.g., needles, lichens), contain biological components (e.g., soil), or are physicochemical in nature (e.g., PRS™ probes, ion exchange resins), all of which are subject to post-sampling biogeochemical processes that may alter sample composition. Proper storage is required to preserve sample integrity, from initial sample acquisition in the field through to ultimate storage in the TEEM sample archive facility.

2.2 Storage & Shipping Plan

Due to the remote nature of the sample locations, and the limited shipping services available in Fort McMurray, it is generally necessary to store samples for periods of up to a few days before they can be shipped to the laboratory(ies). In advance of a field sampling campaign, a storage and shipping plan is to be developed that takes into consideration the volume of samples anticipated each day, the storage requirements for these samples, the size and number of containers needed to ship the samples to the laboratory(ies), the shippers available and any restrictions they may have, and the shipping schedules. The shipping plan must include a process to quickly return empty coolers from the laboratory, otherwise, the capacity of the interim storage facility may be exceeded, particularly in a year when a large number of samples are being collected.

2.3 Storage in the Field

Soil, needle, bark, and lichen samples, PRS™ probes, and ion-exchange resins, are to be placed in coolers immediately after sampling. Pre-frozen (-20°C or colder) freezer packs in sufficient number to prevent any warming of the sample during the remainder of the day are to be included in the cooler, distributed among the samples. The number of coolers and freezer packs required must be determined at the beginning of each field day, such that the appropriate field sample storage capacity is available for samples collected during the field day.

Once full, a cooler should not be opened, as doing so will advance the warming of the samples.

2.4 Interim Storage

At the end of each field day, samples are to be removed from the coolers for inspection, and as required, actions taken to correct sample labelling and/or loss of sample containment (e.g., punctured sample bag). This inspection is to be conducted in a clean environment, under cool, shaded conditions, and by personnel wearing and using appropriate sampling equipment (e.g., nitrile gloves, stainless steel instruments).

Soil samples are to be stored at 4°C or colder. Freezing soil samples (to -20°C) will help maintain samples at a cold temperature for the duration of shipping. Plant and lichen tissue samples are to be stored at -20°C or colder.

2.5 Sample Shipping

Samples are generally shipped in large coolers.

Soil samples are to be cooled to 4°C or colder prior to packaging for shipping. Sufficient ice packs frozen to -20°C (or colder) are to be included in sufficient numbers in each cooler to maintain a sample temperature of 4°C for the duration of the shipping period. Coolers are to be packed in a manner that does not exceed a maximum weight restriction of the chosen shipper.

Plant and lichen tissue samples are to be frozen (-20°C or colder), and packaged with a sufficient number of ice packs (frozen to -20°C or colder) to maintain a maximum temperature inside the cooler of 4°C for the duration of the shipping period.

The chain-of-custody forms for each sample set are to be signed and dated, a copy retained by the TEEM member, inserted into a waterproof, sealable plastic bag, and placed into one of the coolers (a copy of each chain-of-custody form is to be retained). The coolers are to be tightly sealed, including drainage and/or vent ports. Leakage of meltwater from the coolers will cause the internal cooler temperature to rise more quickly, and may cause the shipping company to suspend transport until the leaking liquid can be identified to the shipper's satisfaction. The laboratory and return addresses and phone numbers are to be clearly indicated on each cooler, and the cooler number and the total number of coolers being shipped (e.g., "4 of 10" or "4/10") identified. Waybills, shipping forms, and any other documentation provided by the shipping company are to be retained, and provided to the TEEM Program Manager.

Some samples, including the needle samples collected for epicuticular wax analysis, have specific handling and shipping requirements. These are to be confirmed prior to the sampling program, such that shipping to the laboratory can be completed in a manner that efficiently integrates into the overall field sampling, interim storage and shipping processes.

2.6 Storage at the Laboratory

Proper sample storage at each laboratory is the responsibility of laboratory personnel. This responsibility begins at the time of sample delivery and signing of the chain-of-custody form(s) by the laboratory representative. Each laboratory is to be made aware of the samples that will be sent to them prior to the field sampling campaign, including the types and number of samples, the expected analyses, and the approximate dates on which the samples will be delivered.

Upon arrival at the laboratory, soil samples are to be split into two subsamples, in an approximate 75:25 ratio. The larger subsample is to be immediately set out to dry, or frozen until capacity for drying is available. The remaining 25% of the field moist subsample is to be labelled and double-bagged using sealable, plastic bags, and labelled according to the **SAMPLE LABELLING PROCEDURE (#1)**. This subsample is to remain in field moist condition, for the analysis of available nitrogen and sulphur. This analysis must be initiated immediately, or the subsample frozen until analysis.

2.7 Sample Archive

Residual samples, the amounts remaining after completion of all required laboratory analyses, are to be placed in the TEEM Forest Health Monitoring Program sample archive. The chain-of-custody form is to be signed and dated upon transfer of sample control from the laboratory to TEEM.

Prior to their placement in the archive facility, each sample is to be inspected, and as required, actions taken to correct sample labelling and/or loss of sample containment (e.g., punctured sample bag). This inspection is to be conducted in a clean environment, under cool, shaded conditions, and by personnel wearing and using appropriate sampling equipment (e.g., nitrile gloves, stainless steel instruments).

The majority of samples in the archive will be fully dried, and put into labelled, airtight containers. Some may remain in field moist condition; these are to be stored in a freezer (maximum temperature of -20°C).

PROCEDURE #3
SITE INFORMATION

3.1 Background 15
3.2 Jack Pine Interior Monitoring Site Information 15
3.3 Edge Monitoring Site Information 16

3.1 Background

Topographic information about the site is required at the time of site establishment.

3.2 Jack Pine Interior Monitoring Site Information

Site information for a new interior monitoring site is to be recorded on using TEEM Form 01. Practitioners should complete this form in detail, ensuring that a person who had not visited the site is able to visualize the location of the plot on the terrain. Site information and observations should be from the perspective of the vegetation plot. The open fields in TEEM Form 01 are to be completed as follows:

TEEM Form 01 – Interior Monitoring Site Information

Field(s)	Field Name	Required Information
1 to 5	Site	5-Character site designation
6 to 9	Year of Establishment	The 4-digit year of plot establishment (e.g., “2011”)
10 to 17	Date of Plot Staking	The date of vegetation plot staking, in the format YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
18 to 20	Personnel	Three fields are provided for the full names of the personnel involved
21	Location	A written description of the location, including names, directions and distances to nearest landmarks (creeks, roads, etc.) and comments on the location of the helicopter landing pad relative to the reference stake. All information that is or may be relevant should be included
25 to 33	Latitude	Latitude, in decimal degree format (e.g., “56.12345N”)
34 to 43	Longitude	Longitude, in decimal degree format (e.g., “111.98765W”)
44 to 47	Elevation	A GPS unit with elevation capability is to be used to determine elevation, a four-digit value is used to determine elevation above sea level, to the nearest metre (e.g., 389 m asl would be recorded as “0389”)
48 to 50	Plot Orientation	Three-digit number indicating the compass bearing from true North of the longer axis of the vegetation plot (e.g., 82° would be recorded as “082”)
51 & 52	Slope	The slope in percent is recorded as a two-digit number (e.g., 4% would be recorded as “04”; a flat site would be recorded as “00”)
53 & 54	Aspect	The compass direction of the downward slope at the site in a 2-character format: N-, NE, E-, SE, S-, SW, W-, or NW (cardinal compass points are written as a 2-character code, the second code being a hyphen “-“). A flat site is to be recorded as “FL”

Field(s)	Field Name	Required Information
55	Terrain Position	A one-digit code is used to designate the position of the vegetation plot in relation to the surrounding topography: 1 = top and upper slope, including the convex area on the slope top 2 = midslope, an area of uniform slope between slope top and bottomland, or between a bench and either slope top or bottomland 3 = bench, an area of level terrain with midslope profiles above and below 4 = lower slope, the concave area on the lower part of the slope 5 = flatland, level or near level terrain 6 = bottomland, an area subject to a high water table As jack pine grows on well-drained, sandy soils that are generally elevated above the surrounding terrain, slope position for jack pine sites will usually be designated as 1 (top and upper slope), 5 (flatland), or 3 (bench). Application of any other designation should cause the practitioner to pause in site establishment, and confirm that the plots are being established in the correct location
56	Remarks	Comments and information allowing for clearer understanding of the data and information

3.3 Edge Monitoring Site Information

Site information for a edge monitoring site is to be recorded on using TEEM Form E01. Practitioners should complete this form in detail, ensuring that a person who had not visited the site is able to visualize the location of the plot on the terrain. Site information and observations should be from the perspective of the vegetation plot.

The open fields in TEEM Form E01 are to be completed as follows:

TEEM Form E01 – Edge Monitoring Site Information

Field(s)	Field Name	Required Information
1 to 5	Site	The 5-character site designation
6 to 9	Year of Establishment	The year in which the edge monitoring site is or was established
10 to 17	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-JUL-09”)
18 to 20	Personnel	Three fields are provided for the full name(s) of the personnel conducting the assessment
21	Location	A written description of the location, including names, directions and distances to nearest landmarks (creeks, roads, etc.) and comments on the location of the helicopter landing pad relative to the reference stake. All information that is or may be relevant should be included
21	Location	A written description of the location, including names, directions and distances to nearest landmarks (creeks, roads, etc.) and comments on the location of the helicopter landing pad relative to the reference stake. All information that is or may be relevant should be included
25 to 33	Latitude	Latitude, in decimal degree format (e.g., “56.12345N”)
34 to 43	Longitude	Longitude, in decimal degree format (e.g., “111.98765W”)

Field(s)	Field Name	Required Information
44 to 47	Elevation	A GPS unit with elevation capability is to be used to determine elevation, a four-digit value is used to determine elevation above sea level, to the nearest metre (e.g., 389 m asl would be recorded as “0389”)
48 to 50	Plot Orientation	Three-digit number indicating the compass bearing from true North of the longer axis of the vegetation plot (e.g., 82° would be recorded as “082”)
51 & 52	Slope	The slope in percent is recorded as a two-digit number (e.g., 4% would be recorded as “04”; a flat site would be recorded as “00”)
53 & 54	Aspect	The compass direction of the downward slope at the site in a 2-character format: N-, NE, E-, SE, S-, SW, W-, or NW (cardinal compass points are written as a 2-character code, the second code being a hyphen “-“). A flat site is to be recorded as “FL”
55	Terrain Position	A one-digit code is used to designate the position of the vegetation plot in relation to the surrounding topography: 1 = top and upper slope, including the convex area on the slope top 2 = midslope, an area of uniform slope between slope top and bottomland, or between a bench and either slope top or bottomland 3 = bench, an area of level terrain with midslope profiles above and below 4 = lower slope, the concave area on the lower part of the slope 5 = flatland, level or near level terrain 6 = bottomland, an area subject to a high water table As jack pine grows on well-drained, sandy soils that are generally elevated above the surrounding terrain, slope position for jack pine sites will usually be designated as 1 (top and upper slope), 5 (flatland), or 3 (bench). Application of any other designation should cause the practitioner to pause in site establishment, and confirm that the plots are being established in the correct location
56	Remarks	Comments and information allowing for clearer understanding of the data and information

Full use of the Remarks field is encouraged. Information or comments that may be helpful to staff preparing site documents and reports, to personnel conducting future sampling programs at the site, and observations that might assist in interpretation of sample data should be written into this field (use additional pages if necessary).

PROCEDURE #4
REFERENCE STAKE INSTALLATION & GEO-REFERENCING

4.1 Background 18
4.2 Common Procedure 18
4.3 Interior Stand Site.....18
4.4 Edge Monitoring Site.....19

4.1 Background

Staking and geo-referencing using a combination of GPS coordinates and measurements is required at each site to ensure that personnel charged with monitoring program activities can efficiently and correctly locate each site component. These coordinates and measurements permit the derivation of an accurate site drawing, of value in tracking site status and in evaluating the potential locations of additional monitoring components in a manner that does not compromise those already in place.

4.2 Common Procedure

The reference stake is a 1 m or longer metal stake (e.g., rebar) driven into the ground until about 25 cm remains above the surface. This stake is a permanent installation. Geo-referencing the layout of plots and equipment at the site from this stake will allow re-establishment of the site after a major disturbance, such as a forest fire.

A 50-cm long white plastic, hollow stake (e.g., 2” PVC) is to be driven into the ground to a depth of about 25 cm, leaving about 25 cm of plastic stake above ground. In the centre of this stake, a 1-m length of rebar (or equivalent) is to be driven into the ground until about 25 cm remains above the top of the plastic stake. At the top of the white stake, “WBEA TEEM”, beneath which the site name, is to be written using a black or blue permanent marker.

A GPS unit capable of providing an accuracy of 3 m or better is to be used to record the location of the reference stake. If the GPS unit is capable of averaging, the GPS unit is to be placed on top of or immediately adjacent to the reference stake, and set to average over 20 or more readings. The combination of averaged location readings and a 3 m or better accuracy will ensure that the reference stake can be accurately located and can be found in future years. GPS coordinates are to be recorded in decimal-degree format.

Where plot corners and site features occur close to the reference stake, a 100 m tape measure can be used to measure distances between the reference stake and plot corners and/or site features.

4.3 Interior Stand Site

The site name, in the format of “JPxxx”, where “xxx” is the 3-digit site number, is to be written on the white PVC stake using a black or blue permanent marker.

From the reference stake, a bearing is to be taken (corrected for declination) to the nearest corner of each vegetation and soil plot, to the centre of the off-plot tree area(s), the soil pit, and to any other monitoring system or equipment deployed at the site (e.g., PRS™ probes, ion exchange resins, passive and/or meteorological monitoring tower).

The GPS coordinates (in decimal-degree format) of the corner of each plot nearest the reference stake are to be obtained. From this corner, a compass bearing (corrected for declination) down the long side of the plot is to be obtained.

GPS coordinates (in decimal-degree format) are to be obtained for any other feature relating to the monitoring program (e.g., helipad), and for any other terrain feature that may be relevant to orientation around the site and/or to the monitoring program itself.

4.4 Edge Monitoring Site

Where the edge monitoring site is close to, and within sight of the reference stake at the interior stand monitoring site, the reference stake at the interior site is to be used as the geo-referencing location for both the interior and edge monitoring sites. In this case, both the interior stand site label (“JPxxx”) and the edge monitoring site label (“JExxx”) are to be written on the white plastic stake using a black or blue permanent marker.

Where the edge monitoring site is not sufficiently close to the reference stake at the interior stand monitoring site, a separate reference stake at the edge monitoring site is required. In this instance, the site name, in the format of “JExxx”, where “xxx” is the 3-digit site number, is to be written on the reference stake using a black or blue permanent marker.

The GPS coordinates (in decimal-degree format) of the corner of the edge monitoring plot nearest the reference stake are to be obtained. From this corner, a compass bearing (corrected for declination) down the long side of the plot is to be obtained. Coordinates are also required for any other monitoring system or equipment deployed at the edge monitoring site (e.g., PRS™ probes, ion exchange resins, passive and/or meteorological monitoring tower).

GPS coordinates (in decimal-degree format) are to be obtained for any other feature relating to the monitoring program (e.g., helipad), and for any other terrain feature that may be relevant to orientation around the edge monitoring site and/or to the monitoring program itself.

Where plot corners and site features occur close to the reference stake, a 100 m tape measure can be used to measure distances between the reference stake and plot corners and/or site features.

PROCEDURE #5
TREE NUMBERING & LABELLING

5.1 Background 20
5.2 Common Procedure 20
5.3 Vegetation Plot Trees 20
5.4 Off-Plot Trees 20
5.5 Edge Plot Trees 21

5.1 Background

Vegetation plot trees, off-plot trees, and trees in the edge monitoring plot are to be uniquely marked, with redundancy in marking methods to ensure that at least one mark is clearly visible at all times.

Each time a site is visited, tree markings are to be examined, and as necessary, refreshed or replaced.

5.2 Common Procedure

Trees selected for monitoring are to be labelled using numbered aluminum tags attached to the trunk at about eye level using coated 18 or 24 gauge copper wire. The wire is to be wrapped tightly enough to prevent slipping of the tag, but loose enough to provide space for lateral growth of the tree.

The tree number is to be painted, using tree paint, on two sides of the tree. The number without the preceding alphabetic (e.g., “X” or “E”) and zeros is to be painted onto each side of the trunk (e.g., “1”).

Under the painted numbers, a line 1.3 m above ground level is to be painted onto both sides of the trunk, defining the level at which DBH measurements will be taken.

5.3 Vegetation Plot Trees

A three-digit tree number is assigned to each tree, in the format of “001”. This number is to be etched onto the aluminum tag attached to the tree.

5.4 Off-Plot Trees

The number assigned to off-plot trees is a three-digit number, and preceded with an “X”. Thus, the 10th off-plot tree to be labelled at a site will have the number “X10”. This number is to be etched onto the aluminum tag attached to the tree. The number without the preceding “X” and zeros is to be painted onto each side of the trunk (e.g., “1”).

New numbers are to be assigned to replacement off-plot trees in sequence from the last number used at the site. Numbers are not to be reassigned.

5.5 Edge Plot Trees

A two-digit tree number is assigned to each edge plot tree, in the format of “01”, preceded with an “E”. Thus, the 12th suitable edge monitoring tree is to be assigned the number “E12”. The number assigned to an edge monitoring site tree is permanent. This number is to be etched onto the aluminum tag attached to the tree. The number neither preceding “E” nor the zeros is to be painted onto each side of the trunk (e.g., “1”).

PROCEDURE #6
PUBLIC LANDS ACT DISPOSITIONS

6.1 Common Procedure 22
6.2 Interior Stand Monitoring Site 22
6.3 Edge Monitoring Site.....22

6.1 Common Procedure

At the conclusion of the site establishment program, an application for a disposition under the *Public Lands Act* is to be prepared, and the application submitted through Alberta Sustainable Resource Development's Electronic Disposition System (EDS). The procedures required by AESRD for application for a disposition are specific, and must be followed precisely to avoid a lengthy application process. The application process may be best managed through a consulting contract between WBEA/TEEM and a land agent registered within the EDS system.

While a separate application is generally required for each site, a single application that includes an interior monitoring site and an edge monitoring site that are in close proximity is encouraged. The plot layout and site drawings are required components of the application.

The disposition is granted in the form of a *Public Lands Act* Letter of Authority issued by AESRD. The Wood Buffalo Environmental Association must retain this document; the land agent may also retain a copy of the letter.

6.2 Interior Stand Monitoring Site

The area included in the application is to include the vegetation and soil plots, and the off-plot tree area, plus a minimum buffer of 100 m. Inclusion of a nearby edge monitoring site in a single application is permitted.

6.3 Edge Monitoring Site

The area included in the application is to include the edge monitoring plot, plus a minimum buffer of 50 m. The edge monitoring site may be incorporated into the application for a disposition for a nearby interior stand monitoring site.

PROCEDURE #7
SOIL DESCRIPTION

7.1 Background 23
7.2 Soil Description 23

7.1 Background

The soil exposed in the pit is to be described in sufficient detail that, together with the results of the laboratory analysis of pit samples, the soil can be classified into the appropriate subgroup of the Canadian System of Soil Classification (Soil Classification Working Group, 1998[1]), and assigned the appropriate soil map unit.

7.2 Soil Description

The soil pit information is acquired using TEEM Form 10. The open fields are to be completed as described below:

TEEM Form 10 – Soil Description

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete as appropriate. The first page (Fields 1 to 45) is identified by an “A”; the second page (Fields 1 to 29 and 46 to 58) is identified by a “B”. If soil pit data are collected on different days, complete a separate form for each day. The total number of pages used to collect the soil pit data is to be entered into Field 2 on each of pages “A” and “B”
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	The date, in the format YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19	Location	A written description of the location
20 to 29	Horizon Designation	The horizons exposed in the soil pit are to be named according to Canadian terminology. Ten fields are provided; use additional pages for soils having more than 10 distinct horizons
30 to 33	Depth from Top to Bottom	The depth from the H/A interface to the top and bottom of each horizon is to be recorded to the nearest centimetre
34 to 36	Colour	The colour of the horizon is to be assigned using the Munsell notation (Field 34), the colour name (Field 35), and a note as to whether the soil is moist or dry at the time of examination (Field 36)
37	Texture	Soil texture by feel is to be recorded
38 to 40	Mottles	The presence of mottles in each horizon is to be noted in terms of colour, abundance, and contrast with the soil matrix

[1] Soil Classification Working Group (1998) *The Canadian System of Soil Classification (third edition)*. http://sis.agr.gc.ca/cansis/references/1998sc_a.html

Field(s)	Field Name	Required Information
41 to 45	Structure	Horizon structure is to be noted by grade (Field 41), distinctness (Field 42), class (Field 43), size (Field 44), and type (Field 45)
46	Consistency	By horizon, the resistance of the soil to deformation and the degree of cohesion/adhesion is to be noted
47	Roots	The abundance, size, orientation, distribution and depth of penetration of roots are to be recorded
48	Pores	The abundance, size, orientation, distribution and depth of penetration of pores are to be recorded
49 to 52	Clay Films	Record the frequency, thickness, location and colour of clay films
53	Horizon Boundaries	Record the distinctness of the lower boundary of each horizon, the form of the horizon boundary, and any other characteristic of the boundary
54	Coarse Fragment Content	For the whole pit, provide an estimate in percent (v/v) of the coarse fragment content, and describe the shape, kind, size and name of coarse fragments
55 to 57	Photos	Record photograph number(s), the direction of view of each photograph(s), and other information that will assist in identifying the photograph(s) in the future
58	Remarks	Any other features observed

Full use of the remarks field is encouraged. Information or comments that may be helpful to staff preparing site documents and reports, to personnel conducting future sampling programs at the site, and observations that might assist in interpretation of sample data should be written into this box (use additional pages if necessary).

PROCEDURE #8
SOIL SAMPLE LOCATION & CHECKLIST

8.1 Soil Sample Location 25
8.2 Soil Sample Checklist 25

8.1 Soil Sample Location

The locations of the soil samples taken from within each soil subplot during a routine (6-year) monitoring cycle are to be recorded in TEEM Form 11. The open fields in this form are to be completed as follows:

TEEM Form 11 – Soil Sample Locations

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the last subplot soil sample
3 to 10	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-JUL-09”)
11 to 13	Personnel	Three fields are provided for the full name(s) of the personnel conducting the assessment
14 to 18	Site	5-Character site designation
19	Plot	The 1-digit soil plot number (1 to 4) The field duplicate sample is to be labelled from A to D, with “A” representing soil plot 1, “B” representing soil plot 2, “C” representing soil plot 3, and “D” representing soil plot 4
20	Subplot	The 1-digit soil subplot number (1 to 4). This applies to the field duplicate sample as well
21	Random Number	The 1-digit random number used to select the sampling location
22	Sample Location	The 1-digit number identifying the sample location. This applies to the field duplicate sample as well
23	Adjustment	Enter “Yes” if any adjustment was required in the sample location (i.e., to avoid interference from a tree, to avoid disturbance), or “No”. If “Yes”, describe in Field 24
24	Remarks	Enter remarks specific to the sample location. If “Yes” in Field 23 (Adjustment), enter measurements from subplot boundaries to sample location, and the reason that adjustment was required. Continue in general remarks (Field 25) if additional space is required
25	Remarks	Enter general remarks about soil sampling

8.2 Soil Sample Checklist

Prior to initiating the field program, a sample checklist is to be prepared and used by field personnel to ensure that all required samples are acquired, minimizing the potential for incorrect or inadequate sampling in the field. The checklist for the program in its current configuration is presented below.

Routine Soil Monitoring Program – Soil Sample Checklist (per Site)

Depth	Number of Plots (a)	Number of Subplots (b)	Number of Samples by Depth (a x b)	Total Number of Samples
Site Establishment Year				
LFH	4	4	16	85
0 to 5 cm	4	4	16	
5 to 15 cm	4	4	16	
15 to 30 cm	4	4	16	
30 to 50 cm	4	4	16	
Field duplicate LFH	1	1	1	
Field duplicate 0 to 5 cm	1	1	1	
Field duplicate 5 to 15 cm	1	1	1	
Field duplicate 15 to 30 cm	1	1	1	
Field duplicate 30 to 50 cm	1	1	1	
Routine Monitoring Year				
LFH	4	4	16	68
0 to 5 cm	4	4	16	
5 to 15 cm	4	4	16	
15 to 30 cm	4	4	16	
Field duplicate LFH	1	1	1	
Field duplicate 0 to 5 cm	1	1	1	
Field duplicate 5 to 15 cm	1	1	1	
Field duplicate 15 to 30 cm	1	1	1	

PROCEDURE #9
SOIL SAMPLE PREPARATION

9.1 Division of Soil Samples 27
9.2 Soil Sample Drying 27
9.3 Soil Sample Grinding 27
9.4 Moisture Correction 28
9.5 Weighing Samples for Analysis 28

9.1 Division of Soil Samples

Upon receipt at the laboratory, soil samples are to be split into two subsamples in an approximate 75:25 ratio. The larger of the subsamples will be dried, while the smaller is to be reserved in field fresh status for the analyses of soluble nutrients (sulphur and nitrogen). The field fresh subsample is to be stored at 4°C, unless analyses will be substantially delayed, in which case, storage in a freezer (-20°C) is required.

9.2 Soil Sample Drying

The larger of the soil subsamples are to be dried, preferably in a dedicated drying room. Oven drying is not required.

Samples are transferred to a drying tray, and the paper label is to be placed in or under the drying tray. After drying, the 75% subsample is to be passed through a 2 mm sieve. Lumps of soil may be broken by hand. This ground material is to be put into a sturdy plastic container, labelled according to the **SAMPLE LABELLING PROCEDURE (#1)**, and sealed until required for analysis.

9.3 Soil Sample Grinding

9.3.1 Field Fresh Samples

Care must be taken to obtain a representative, field fresh sample. In a cold room, LFH samples are to be roughly homogenized (10 to 20 sec.) in a commercial stainless steel food processor (chilled), taking care to prevent the samples from warming or losing moisture. Once sufficient field fresh, ground sample material is obtained, the remainder is to be dried and milled.

9.3.2 Dried LFH and Mineral Soil Samples

Dried LFH material is to be ground to pass through a 2 mm screen using a Wiley mill equipped with hardened tool steel blades. Care must be taken to ensure mineral component does not enter the mill otherwise damage may result. Ground material is to be put into plastic container, leaving sufficient headspace to permit proper mixing of the material without opening the container. The container is to be labelled according to the **SAMPLE LABELLING PROCEDURE (#1)**, and sealed until required for analysis.

Grinding to a 100 mesh particle size is required for some of the analyses. A subsample of the 2 mm sample is to be obtained in a manner that is representative of the sample, and ground using either a high speed centrifugal mill, ball mill or ring grinder. The ground material is to be put into an appropriate container (plastic vial), leaving sufficient headspace to permit proper mixing of the material without opening the container. The container is to be labelled according to the **SAMPLE LABELLING PROCEDURE (#1)**, and sealed until required for analysis.

9.4 Moisture Correction

Analytical results are generally to be reported on a moisture-corrected basis. To correct for sample moisture, a representative 40 g (±0.01g) subsample is to be taken and dried in an oven set to 105°C, for 24 to 48 hours. Upon removal from the oven, the container is to be tightly sealed, cooled and weighed to the same level of precision (±0.01g). From these two weights, the percent water content is to be calculated, and the results of each of the analyses performed on this sample are to be corrected for this water content.

9.5 Weighing Samples for Analysis

Weighing of samples is conducted on a balance with the accuracy and precision required for the analysis. The accuracy and precision requirements of each analysis are stipulated in the individual analytical procedures.

PROCEDURE #10
SOIL TEXTURE ANALYSIS

10.1. Background 29
10.2. Reagents 29
10.3. QA/QC Controls 29
10.4. Analysis 29
10.5. Calculations 30

10.1 Background

Each mineral soil horizon sampled from the soil pit is to be analysed for soil texture using the Bouyoucos Hydrometer procedure (Kalra and Maynard, 1991[2]; Kroetsch and Wang, 2008[3]), as applied to sandy soil samples of up to 5% of organic matter.

10.2 Reagents

1. Water of ASTM Type II quality, or better.
2. Sodium hexametaphosphate at 50g/L, adjusted to pH 8.2 using $Na_2(CO_3)_2$.
3. Commercial amyl alcohol defoaming agent.

10.3 QA/QC Controls

1. Blank (1 per batch): leave a dispersion cup empty, process as if the cup contains a soil sample.
2. Reference standard (1 per batch): transfer 40 g (±0.01 g) of a sample of known sand, silt and clay content into a dispersion cup.
3. Replicate (1 per batch): from one randomly selected sample within the batch, transfer 40 g (±0.01 g) of 2 mm air-dried soil into a second dispersion cup.

10.4 Analysis

1. Transfer 40 g (±0.01 g) 2 mm air-dried mineral soil to a dispersion cup.
2. Add 200 mL water.
3. Add 100 mL sodium hexametaphosphate solution and stir on the milkshake machine for 15 min.
4. Transfer the soil suspension quantitatively to the sedimentation cylinder.
5. Add water to bring the sample volume to 1 L (the blank is to consist of 100 mL hexametaphosphate solution and 900 mL water).

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[2] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 9. Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

[3] Kroetsch D, Wang, C (2008) Particle size distribution. Section 55. *In* Carter MR, Gregorich EG (2008) *Soil Sampling and Methods of Analysis, Second Edition.* Canadian Society of Soil Science, CRC Press, Boca Raton FL. pp 713-725

6. Cover the cylinder with a watch glass and let it stand overnight to equilibrate to room temperature or in a water bath that holds temperature to between 20°C and 25°C, on a vibration-free bench.
7. Insert the plunger close to the bottom of the cylinder and stir the suspension vigorously for 2 min. by moving the plunger up and down the whole length of the column (about 25 strokes), in order to loosen sediment settled on the bottom of the cylinder. Move the plunger cautiously near the top of the cylinder to avoid spilling the contents. It is important not to remove plunger out of the suspension or bubbles may form, disrupting sedimentation. Finish stirring with two or three slow, smooth strokes.
8. Remove the plunger, tipping it slightly to remove adhering drops of suspension.
9. Immediately lower a hydrometer gently into the suspension.
10. Add a couple of drops of amyl alcohol if the surface of the suspension is covered with foam.
11. Take the hydrometer reading (top of the meniscus) exactly 40 sec. after the completion of stirring.
12. Remove the hydrometer. Determine the temperature of the suspension at about 5 cm depth. Clean the hydrometer with water for the following suspensions.
13. Let the cylinder stand undisturbed for 2 hr.
14. Take hydrometer readings. Use the same hydrometer for all cylinders.

10.5 Calculations

1. Corrected hydrometer readings are obtained by subtracting the blank reading.
2. Calculate Sand, Silt and Clay contents as follows:

$$\text{Sand}(\%) = 100 - [(R_{40s} - R_{bl}) \times (100/\text{Moisture-corrected Sample Weight})]$$

$$\text{Clay}(\%) = (R_{2h} - R_{bl}) \times (100/\text{Moisture-corrected Sample Weight})$$

$$\text{Silt}(\%) = 100 - (\text{Sand}(\%) + \text{Clay}(\%))$$

R_{bl} = hydrometer reading of the blank

R_{40s} = hydrometer reading at 40 sec.

R_{2h} = hydrometer reading at 2 hr

PROCEDURE #11
SOIL PH ANALYSIS

11.1 Background 31
11.2 Reagents 31
11.3 QA/QC Controls 31
11.4 Organic (LFH) Material 31
11.5 Mineral Soil Material 31
11.6 Analysis 32

11.1 Background

Samples taken from the LFH horizon and each mineral soil horizon are to be analysed for soil pH. Litter and mineral soil samples used for this analysis are to be air-dried. The pH of a soil sample is measured using the procedure based on Kalra and Maynard (1991[4]).

11.2 Reagents

1. Water of ASTM Type I quality
2. 0.01 M $CaCl_2$, with a pH between 5.0 and 6.5 (adjusted with $Ca(OH)_2$ or HCl as required).

11.3 QA/QC Controls

1. Commercial standards: analyse a minimum of one each of pH 4 and 7 standards per sample batch.
2. Reference standard (1 per batch): transfer 10 g (±0.01g) of a soil of known pH into a beaker.
3. Replicate (1 per batch): from one randomly selected sample within the batch, transfer 10 g (±0.01 g) of 2 mm air-dried soil into a second dispersion cup.

11.4 Organic (LFH) Material

1. Weigh 10 g (±0.01g) of air-dried, 2 mm LFH material into a beaker.
2. Add 40 mL 0.01 *M* $CaCl_2$ solution.

11.5 Mineral Soil Material

1. Weigh 10 g (±0.01g) of 2 mm air-dried mineral soil into a beaker.

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[4] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Chapter 7(ii). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

2. Add 20 mL of 0.01 *M* $CaCl_2$ solution.

11.6 Analysis

1. Allow soil to absorb $CaCl_2$ solution without stirring.
2. Thoroughly stir for 10 sec., then stir for 10 seconds five times over 30 min.
3. Allow suspension to settle for 30 min.
4. Measure pH by immersing a combination electrode in the supernatant solution.
5. Record pH value when the reading has stabilized.

PROCEDURE #12
SOIL ELECTRICAL CONDUCTIVITY ANALYSIS

12.1 Background 33
12.2 Reagents 33
12.3 Organic (LFH) Soil 33
12.4 Mineral Soil 33
12.5 QA/QC Controls 34
12.6 Analysis 34

12.1 Background

Soil salinity is to be assessed by measuring the electrical conductivity (EC) of a soil extract (Miller and Curtin, 2008[5]).

12.2 Reagents

1. Water of ASTM Type I quality, or better

12.3 QA/QC Controls

1. Commercial standard (1 per batch): standard of known EC.
2. Reference standard (1 per batch): transfer 10 g (±0.01g) of LFH, or 20 g (±0.01g) of 2 mm air-dried mineral soil, of known EC into a 50 mL polypropylene centrifuge tube. Add water of a volume appropriate for the sample (defined above).
3. Replicate (1 per batch): from one randomly selected sample within the batch10 g (±0.01g) of LFH, or 20 g (±0.01g) of 2 mm air-dried mineral soil into a 50 mL polypropylene centrifuge tube. Add water of a volume appropriate for the sample (defined above).

12.4 Organic (LFH) Soil

1. Weigh 10 g (±0.01g) of air-dried, 2 mm LFH material into a 50 mL polypropylene centrifuge tube.
2. Add sufficient water to achieve 1:4 soil to water (w/v) ratio.

[5] Miller JJ, Curtin D (2008) Electrical conductivity and soluble ions. Section 15. *In* Carter MR,Gregorich E. G. (eds) *Soil Sampling and Methods of Analysis, Second Edition*, Canadian Society of Soil Scientists

12.5 Mineral Soil

1. Weigh 20 g (±0.01g) of 2 mm air-dried mineral soil into a 50 mL polypropylene centrifuge tube.
2. Add sufficient water to achieve 1:2 soil to water (w/v) ratio.

12.6 Analysis

1. Shake the centrifuge tubes for 1 hr.
2. Decant, and centrifuge supernatant at 2,000 g for 10 min.
3. If analysis cannot be completed immediately after centrifugation, store filtrate at 4°C. Allow stored samples to warm to room temperature before analysis.
4. Read conductivity of extracts using EC probe and meter. Report results in units of S m^{-1} or dS m^{-1}

PROCEDURE #13
SOIL CATION EXCHANGE CAPACITY ANALYSIS

13.1 Background 35
13.2 Reagents: 35
13.3 QA/QC Controls 35
13.4 Organic (LFH) Soil 35
13.5 Mineral Soil 36
13.6 NH_4Cl Extraction for Exchangeable Cations 36
13.7 Ethanol Wash 36
13.8 NaCl Extraction 37
13.9 Calculations 37

13.1 Background

The CEC of a soil sample is to be measured in an extract of the soil, based on the methods of Kalra and Maynard (1991[6]) and Skinner et al. (2001[7]).

13.2 Reagents:

1. Water of ASTM Type I quality.
2. 1.0 M NH4Cl, unbuffered.
3. 95% USP ethyl alcohol (ethanol).
4. 10% NaCl, acidified to 0.005 M using HCl.

13.3 QA/QC Controls

4. Standard solutions for calibration of the segmented flow analyser.
5. Reference standard (1 per batch): transfer 0.5 g (±0.01 g) of LFH, or 2.5 g (±0.01 g) of 2 mm air-dried mineral soil, of known CEC.
6. Blank (1 per batch): an sample tube without soil material added.
7. Replicate (1 per batch): from one randomly selected sample within the batch; 10 g (±0.01 g) of LFH, or 20 g (±0.01 g) of 2 mm air-dried mineral soil.

13.4 Organic (LFH) Soil

1. Weigh 0.50 g (±0.01 g) of air-dried, 2 mm LFH material.

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[6] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 15(ii). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

[7] Skinner MF, Zabowski D, Harrison R, Lowe A, Xue D (2001) Measuring the cation exchange capacity of forest soils. Comm. Soil Sci. Plant Anal. 32:1751-1754

13.5 Mineral Soil

1. Weigh 2.50 g (±0.01 g) of air-dried, 2 mm mineral soil.

13.6 NH_4Cl Extraction for Exchangeable Cations

1. Place a filter pad on top of the filter frit, within the sample tube, and close the tube.
2. Place the soil onto the filter pad, levelling if necessary.
3. Connect sample tube to upper disk of extractor.
4. Weigh lower collection syringe and plunger assembly (±0.01 g), and connect assembly to lower disk of extractor.
5. Attach sample tube assembly to the collection syringe.
6. Fill sample tube to the 22.5 mL mark with 1.0 *M* NH_4Cl.
7. Stir, then rinse the stirring rod with NH_4Cl, bringing total volume to 25 mL.
8. Let stand for 20 min.
9. Extract rapidly (over 15 min) until about 15 mL of the solution has entered the collection syringe.
10. Wash the walls of the sample tube with extraction solution and top up to 45 mL.
11. Set extractor on 12-hour setting and leave it running overnight.
12. The next morning, turn off the extractor and pull the plungers down as far as the extractor will allow. Disconnect collecting syringes from rubber connectors and sample tubes.

Begin ethanol wash procedure (below), complete next two steps while ethanol wash is in progress.

13. Weigh syringe(s) containing the NH_4Cl extract (±0.01 g).
14. Mix the NH_4Cl extract thoroughly, then either immediately initiate the **SOIL EXCHANGEABLE CATIONS ANALYSIS (#14)**, or place extract into storage (4°C).

13.7 Ethanol Wash

1. Reset extractor to starting position.
2. Attach new collection syringes to the sample tubes.
3. Rinse sides of sample tubes with 95% ethanol, filling tubes to the 22.5 mL mark.
4. Stir and rinse, bringing total volume to 25 mL.
5. Let stand for 20 min.
6. Extract rapidly (half-hour setting) until about 15 mL of ethanol have drained into the collection syringe. Turn off extractor.

7. Wash down sides of sample tubes and top up with ethanol to 45 mL.
8. Set extractor for 1.5 to 1.75 hr.
9. After extractor stops, turn off switch, pull plungers down, and remove syringes. Discard ethanol wash.
10. Remove reservoir tube and return extractor to starting position.
11. Reattach collection syringes to sample tube and add about 45 mL ethanol. Do not stir. Immediately extract again for approximately 45 min.
12. When extractor has stopped, remove collection syringes and discard ethanol wash.

13.8 NaCl Extraction

1. Attach pre-weighed (±0.01 g) collection syringes.
2. Add 20 mL 10% NaCl solution to sample, stir.
3. Rinse with NaCl solution, bring total volume to the 25 mL mark.
4. Extract rapidly (half-hr setting), until about 15 mL has entered the collection syringe.
5. Wash sample tube sides with NaCl, bring volume to 15 mL mark, fill reservoir to 30 mL mark (total NaCl volume used should be about 60 mL).
6. Set extractor for 1.5 hr.
7. When completed, remove and weigh collection syringes (±0.01 g).
8. Mix NaCl extract thoroughly, transfer to 50 mL centrifuge tube.
9. If samples cannot be analysed within 24 hr, freeze samples. For analysis, samples must be warmed to room temperature.
10. Analyse colourimetrially using a Segmented Flow Analyzer.

13.9 Calculations

Results are to be reported in units of $cmol^+/kg$, taking into account the reported sample reading from the flow analyser corrected for the sample blank, cation charges, molecular weights, volume of extractant (converted from weights), weight of sample, and the dilution factor.

PROCEDURE #14
SOIL EXCHANGEABLE CATIONS ANALYSIS

14.1 Background 38
14.2 QA/QC Controls 38
14.3 Analysis 38
14.4 Calculations 38

14.1 Background

The NH_4Cl extract containing the base cations that was set aside during the analysis of cation exchange capacity (**CATION EXCHANGE CAPACITY PROCEDURE (#13)**) is to be analysed for the concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Mn^{2+}, Al^{3+}, and Fe^{2+}.

14.2 QA/QC Controls

Standards of appropriate concentrations for each of the analyte ions are to be used to calibrate the ICP-AES instrument.

14.3 Analysis

The NH_4Cl extract containing the base cations generated during the initial stages of extraction for Cation Exchange Capacity is to be analysed using ICP-AES for the concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Mn^{2+}, Al^{3+}, and Fe^{2+}. Samples stored at 4°C are to be warmed to room temperature prior to analysis.

14.4 Calculations

Results are to be reported as cation concentration ($cmol^{+}/kg$), taking into account the reported ppm result from the ICP-AES instrument, cation charges, molecular weights, volume of extractant (converted from weights), weight of sample, and the dilution factor.

PROCEDURE #15
SOIL BC:AL RATIO CALCULATION

15.1 Background 39
15.2 Calculation 39

15.1 Background

The BC:Al ratio is a calculated value, based on the molar charge (cmol^{+}/kg) of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and Al^{3+} in the soil exchangeable cation extract that was analysed by ICP-AES (**SOIL EXCHANGEABLE CATION ANALYSIS PROCEDURE (#14)**).

15.2 Calculation

The BC:Al is derived by dividing the sum of the molar charges (cmol^{+}/kg) of Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} by the molar charge (cmol^{+}/kg) of Al^{3+}. The ratio is unitless.

PROCEDURE #16
SOIL BASE SATURATION PERCENTAGE CALCULATION

16.1 Background 40
16.2 Calculation 40

16.1 Background

The Base Saturation Percentage (BS%) is derived from the exchangeable cation concentration divided by the cation exchange capacity.

16.2 Calculation

The BS% is calculated as the sum of molar charge ($cmol^+/kg$) of the exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) measured by ICP-AES (**SOIL EXCHANGEABLE CATIONS ANALYSIS PROCEDURE (#14)**) divided by the CEC ($cmol^+/kg$) (**SOIL CATION EXCHANGE CAPACITY PROCEDURE (#13)**), multiplied by 100.

The ratio is expressed as a percentage.

PROCEDURE #17
TOTAL SULPHUR, NITROGEN & CARBON ANALYSIS

17.1 Background 41
17.2 QA/QC 41
17.3 Analysis 41
17.4 Calculations 42

17.1 Background

Total sulphur, nitrogen and carbon content in soils, plant tissues (needles, bark) and lichens are to be measured using dry combustion (Skjemstad and Baldock, 2008[8]). Samples are flash combusted under helium in the presence of oxygen and the resulting gases (SO_2, NO_2 and CO_2) are separated and analysed. Preference is given to an instrument capable of providing simultaneous measurement of sulphur, nitrogen and carbon. Instruments capable of performing one analysis alone, or two of the analyses simultaneously, are acceptable.

17.2 QA/QC

1. Appropriate commercial standards, matching the sample matrix as closely as possible.
2. Reference standard (1 per batch): an appropriate amount of LFH or mineral soil, plant tissue, or lichen tissue, of known total sulphur, nitrogen and carbon content.
3. Replicate (1 per batch): from one randomly selected sample within the batch.

17.3 Analysis

The amount of sample to be weighed into a combustion crucible depends on the instrument. Micro-analysers require less sample material (e.g., 20 mg) than do macro-analysers (e.g., 500 mg). To ensure sample homogeneity, micro-analysers require a finely ground (<100 mesh) sample.

1. Weigh into a combustion crucible an appropriate amount of finely ground 100 mesh) sample material
2. Where required, add catalyst to the crucible.
3. Follow instrument procedures for combustion and analysis.

[8] Skjemstad JO, Baldock JA (2008) Total and Organic Carbon. Chapter 21 *In* Carter MR and Gregorich EG (Eds.) *Soil Sampling and Methods of Analysis* 2nd Edition, Canadian Society of Soil Science, CRC Press Boca Raton, FL. pp 225-237

17.4 Calculations

The concentration of sulphur, nitrogen and carbon in the sample are to be recorded in μg S/g, μg N/g, and μg C/g, all on a dry weight (moisture-corrected) basis.

Details regarding the analytical, instrumented process, and instrument settings are to be reported with the data.

PROCEDURE #18
SOIL C:N CALCULATION

18.1 Background 43
18.2 Calculation 43

18.1 Background

The C:N ratio is indicative of nitrogen loading to soils.

18.2 Calculation

The carbon to nitrogen ratio (C:N) is calculated by dividing the carbon content of a sample by the nitrogen content, both derived from the results of the dry combustion analysis (**SOIL CARBON, NITROGEN & SULPHUR ANALYSIS PROCEDURE (#17)**).

The ratio is unitless.

PROCEDURE #19
SOIL COMPLEXED ALUMINUM & IRON ANALYSIS

19.1 Background 44
19.2 Reagents 44
19.3 QA/QC Controls 44
19.4 Pyrophosphate Extraction 45
19.5 Dithionate Extraction 45
19.6 Analysis 45

19.1 Background

The extractions and analysis are based on Courchesne and Tunnel (2008[9]). The pyrophosphate extraction yields an extract containing organically-complexed Al and Fe, while the dithionate extraction yields an extract containing bulk (total) Al and Fe, within mineral soils. These results are used in the classification of the soil.

Both procedures below require that the mineral soil sample to be ground to pass through a 100 mesh (0.15 mm) screen.

19.2 Reagents

1. Water of ASTM Type I quality
2. 0.1 M sodium pyrophosphate
3. 0.68 M sodium citrate
4. Dithionite (sodium hydrosulfite; $Na_2S_2O_4$) crystal

19.3 QA/QC Controls

8. Standard solutions for calibration of the AAS or ICP instrument.
9. Reference standard (1 per batch): 0.5 g (±0.01 g) of LFH, or 2.5 g (±0.01 g) of 2 mm air-dried mineral soil, of known Al and Fe content.
10. Blank (1 per batch): a centrifuge tube without soil material added.
11. Replicate (1 per batch): from one randomly selected sample within the batch.

[9] Courchesne F, Tunnel M-C (2008) Extractable Al, Fe, Mn, and Si. Section 26. *In* Carter MR, Gregorich EG (Eds) *Soil Sampling and Methods of Analysis, Second Edition*. Canadian Society of Soil Science, CRC Press Boca Raton, FL. pp 307-315

19.4 Pyrophosphate Extraction

5. Weigh 300 mg (±1 mg) of ground (100 mesh) soil into a 50 mL screw-cap plastic centrifuge tube (use 1 g for samples low in extractable Fe and Al).
6. Add 30 mL of 0.1 *M* sodium pyrophosphate solution.
7. Stopper tightly and gently shake overnight.
8. Decant 2 mL into a small centrifuge tube, centrifuge at 20,000 g for 10 min.
9. Dilute the clarified sample 1:10 with water.

19.5 Dithionate Extraction

1. Weigh 500 mg (1 mg) of ground (100 mesh) soil into a 50 mL screw-cap plastic centrifuge tube.
2. Add 25 mL of 0.68 *M* sodium citrate solution.
3. Add approximately 0.4 g of dithionite (sodium hydrosulfite: $Na_2S_2O_4$), using a calibrated scoop.
4. Stopper tightly and gently shake overnight.
5. Decant 2 mL into a small centrifuge tube, centrifuge at 500 g to 2,000 g for 20 min.
6. Dilute the clarified sample 1:10 with water.

19.6 Analysis

Determine Fe and Al in the pyrophosphate and dithionate extracts using AAS or ICP.

PROCEDURE #20
SOIL SOLUBLE CATIONS ANALYSIS

20.1 Background 46
20.2 Reagents 46
20.3 QA/QC 46
20.4 Organic (LFH) Soil Sample Preparation 46
20.5 Mineral Soil Sample Preparation 46
20.6 Extraction & Analysis 47

20.1 Background

The concentrations of the nutrient base cations in soil solution represent the pool of ions available to plants through root uptake. The analysis of soluble Na^+, K^+, Ca^{2+}, and Mg^{2+} is based on the methods of Kalra and Maynard (1991[10]) and Miller et al. (2008[11]).

20.2 Reagents

1. Water of ASTM Type I quality

20.3 QA/QC

1. Standard solutions for calibration of the ICP instrument.
2. Reference standard (1 per batch): 5 g (±0.01 g) of LFH, or 10 g (±0.01 g) of mineral soil, of known Na^+, K^+, Ca^{2+}, and Mg^{2+} content.
3. Blank (1 per batch): a polypropylene tube without soil material.
4. Replicate (1 per batch): from one randomly selected sample within the batch.

20.4 Organic (LFH) Soil Sample Preparation

1. Weigh 5 g of 2 mm air-dry LFH material into a plastic polypropylene tube.
2. Add sufficient water to achieve 1:8 soil to water ratio; add the water slowly so as to avoid overflowing of the tube.

20.5 Mineral Soil Sample Preparation

1. Weigh 10 g of 2 mm air-dry soil into a polypropylene tube.
2. Add sufficient deionized water to achieve 1:2 soil to water ratio.

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[10] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 8(iv). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

[11] Miller JJ, Curtin D (2008) Electrical Conductivity and Soluble Ions. Section 15. *In* Carter MR, Gregorich EG *Soil Sampling and Methods of Analysis, Second Edition.* Canadian Society of Soil Science, CRC Press Boca Raton, FL. pp 141-159

20.6 Extraction & Analysis

1. Shake tubes at moderate speed for 1 hr.
2. Decant into a clean tube, centrifuge at 2,000 g for 15 min.
3. Store clarified sample at 4°C if analyses cannot be run immediately after centrifugation.
4. Filter using a 0.45 µm micropore filter, or pass through a serum separator.
5. Determine Na^+, K^+, Ca^{2+}, Mg^{2+} concentrations using ICP
6. Report concentrations in units of $cmol^+/kg$.

PROCEDURE #21
SOIL SOLUBLE NITROGEN ANALYSIS

21.1 Background **48**
21.2 Reagents **48**
21.3 QA/QC **48**
21.4 Organic (LFH) Soil Sample Preparation **49**
21.5 Mineral Soil Sample Preparation **49**
21.6 Extraction & Analysis **49**
21.7 Calculations **49**

21.1 Background

The majority of nitrogen in the soil that is available to plants as nitrate (NO_3^-) and ammonium (NH_4^+). The analysis of the nitrate (NO_3^-) and ammonium (NH_4^+) levels in soil is based on the methods of Carter and Gregorich (2008[12]) and Kalra and Maynard (1991[13]).

There is some flexibility in this method, including the weights of samples analysed, the amount of extractant, and the instrument used in the quantification of NO_3^- and NH_4^+. Standard laboratory practices permit these variances, however, all variances employed must be reported with the results to the TEEM Program Manager.

21.2 Reagents

21 Water of ASTM Type I quality

22 2 *N* KCl

21.3 QA/QC

5. Standard solutions for calibration of the analytical instrument.
6. Reference standard (1 per batch): 1 g (±0.01 g) of LFH, or 2.5 g (±0.01 g) of mineral soil, of known Na^+, K^+, Ca^{2+}, and Mg^{2+} content.
7. Blank (1 per batch): a polypropylene tube without soil material.
8. Replicate (1 per batch): from one randomly selected sample within the batch.

---

[12] Maynard DG, Kalra YP, Crumbaugh JA (2008) Nitrate and Exchangeable Ammonium Nitrogen. Section 6.2. *In* Carter MR, Gregorich EG (2008) *Soil Sampling and Methods of Analysis, Second Edition*. Canadian Society of Soil Scientists

[13] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 11(ii). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

21.4 Organic (LFH) Soil Sample Preparation

Care must be taken to obtain a representative, field fresh sample (**Soil Sample Preparation Procedure #9**).

1. Weigh 1.0 g of field moist LFH into a 50 mL polypropylene tube.

21.5 Mineral Soil Sample Preparation

1. Weigh 2.5 g (mineral) of field moist mineral soil into a 50 mL polypropylene tube.

21.6 Extraction & Analysis

1. Add 25 mL 2 *N* KCl solution.
2. Shake sample for 1 hr.
3. Decant into a centrifuge tube, centrifuge at 2,000 g for 15 min.
4. If analysis cannot take place within 24 hr after extraction, refrigerate (4°C) clarified samples until analysis can be completed.
5. Determine NH_4^+ and NO_3^- in extracts using colourimetric determination, segmented flow analyser equipped with a dialysis membrane (to ensure suspended solids and coloured co-extractives don't interfere), or ion selective electrode.

21.7 Calculations

The concentrations of NH_4^+ and NO_3^- in the sample are to be expressed in g/kg soil, on a dry-weight (moisture-corrected) basis.

PROCEDURE #22
SOIL SOLUBLE PHOSPHORUS ANALYSIS

22.1 Background 50
22.2 Reagents 50
22.3 QA/QC 51
22.4 Soil Sample Preparation 51
22.5 Extraction & Analysis 51
22.6 Calculations 52

22.1 Background

Soluble phosphorus in soil samples is to be determined according to the Bray P-1 procedure as described in United States Department of Agriculture (2004[14]), which is based on Bray and Krutz (1945[15]). This procedure has been most successful on acid soils (Olsen and Sommers, 1982[16]). The acid solubilizes calcium and aluminum phosphates, and partially extracts iron phosphates compounds. Aluminum in solution forms a complex with the NH_4F, limiting re-adsorption of phosphorus on iron oxides (Kuo, 1996[17]).

22.2 Reagents

1. Water of ASTM Type I quality
2. Bray-1 extracting solution:
    1. Dissolve 8.88 g of NH_4F in 4 L of water.
    2. Add 200 mL 1.0 *N* HCL.
    3. Dilute to 8 L using reverse osmosis-deionized water.
    4. Confirm pH of 2.60 (±0.05).
3. Colour Reagent:
    1. Dissolve 2.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}$•$4H_2O$] in 800 mL water in a 1 L volumetric flask.
    2. Add 0.025 g antimony potassium tartrate.
    3. Add 20 mL 0f 54% H_2SO_4.
    4. Add 2 g ascorbic acid.
    5. Bring to 1 L with water.

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[14] United States Department of Agriculture (2004) *Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42*. Version 4.0. November 2004. Procedure 4D3b1. pp 234-239

[15] Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci. 59:39-45

[16] Olsen SR, Sommers LE (1982) Phosphorus. *In* Page AL, Miller RH, Keeney DR (eds.) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI. p. 403-430

[17] Kuo S (1996) Phosphorus. *In* Sparks DL (ed.) *Methods of Soil Analysis. Part 3. Chemical Methods*. No. 5. ASA and SSSA, Madison, WI. p. 869-919

4. Standard phosphorus stock solution (prepare weekly):
    1. Dissolve 1.0985 g of oven-dried (2 hr at 105°C) potassium dihydrogen phosphate (KH_2PO_4) in Bray-1 extractant (about 150 mL) in a 250 mL volumetric flask.
    2. Bring to 250 mL with extracting solution.

22.3 QA/QC

5. Phosphorus calibration solutions (prepare weekly) by dilution of the standard phosphorus stock solution to prepare 0.0, 0.1, 0.5, 1.0, 5.0 and 10.0 mg/L calibration curve standards.
6. Reference standard (1 per batch): 2.5 g (±0.001) of LFH or mineral soil (both ground to 2 mm particle size), of known soluble phosphorus content.
7. Blank (1 per batch): a polypropylene tube without soil material.
8. Replicate (1 per batch): from one randomly selected sample within the batch.

22.4 Soil Sample Preparation

9. Weigh 2.5 g (±0.001) of 2 mm, air-dry soil (either LFH or mineral soil) into a 50 mL centrifuge tube.

22.5 Extraction & Analysis

10. Add 25 mL of Bray-1 extracting solution to centrifuge tube.
11. Place tube on shaker, shake for 15 min at medium speed.
12. Centrifuge at 2,000 g for 10 min.

Steps 2 (shaking time and speed) and 3 (centrifugation) must be rigidly standardized (within a laboratory, among laboratories, and from year to year). As long as sample and extractant are in contact, extraction continues, therefore, deviations in shaker and/or centrifuge times will cause variability within the available phosphorus dataset.

13. If analysis cannot take place within 24 hr after extraction, refrigerate (4°C) clarified samples until analysis can be completed.
14. Dilute 0.15 mL sample (supernatant) with 3.6 mL of colour reagent using a digital dilutor
15. Completely mix the sample and colour reagent.
16. Allow 30 min for colour development.
17. Analyse using a segmented flow analyser (use of a dialysis membrane is recommended if analyte concentration is high enough and particulates are of concern), or UV/Vis spectrophotometer set at an absorbance of 882 nm.

22.6 Calculations

Convert phosphorus concentration (mg/L) in the extract to soil phosphorus concentration (g P/kg) as follows:

Soil P (g/kg) = [(A x B x C x R x 1000 x 1000)/E] where:

A = instrument reading (mg/L)
B = extract volume (L)
C = dilution, if performed
R = field-moist/oven-dry ratio (**SOIL SAMPLE PREPARATION PROCEDURE (#9)**)
E = sample weight (g)

Report soluble phosphorus concentrations to the nearest 0.0001 g P/kg soil.

PROCEDURE #23
SOIL INORGANIC SULPHUR ANALYSIS

23.1 Background 53
23.2 Reagents 53
23.3 QA/QC 53
23.4 Organic (LFH) Soil Sample Analysis 54
23.5 Mineral Soil Sample Analysis 54
23.6 Calculations 54

23.1 Background

Sulphur, in the form of sulphate (SO_4^{2-}), is a principal anion in acid deposition, and SO_4^{2-} is generally the primary form of inorganic sulphur (S_i) found in mineral soils.

The analytical procedures for LFH (Kalra and Maynard, 1991[18]) and mineral soil (Kalra and Maynard, 1991[19]) samples differ. The LFH material must be in field-moist condition.

23.2 Reagents

23 Water of ASTM Type I quality

24 0.01 N NH_4Cl

25 500 mg L^{-1} $Ca(H_2PO_4)_2$

23.3 QA/QC

9. Standard solutions for calibration of the analytical instrument.
10. Reference standard (1 per batch): an amount of field-moist sample approximately equivalent to 2 g (weighed to ±0.01 g) of LFH, or 2 g (±0.01 g) of air dry mineral soil, of known inorganic sulphur content.
11. Blank (1 per batch): a polypropylene tube without soil material.
12. Replicate (1 per batch): from one randomly selected sample within the batch.

[18] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 14(i). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

[19] Kalra YP, Maynard DG (1991) *Methods Manual for Forest Soil and Plant Analysis. Information Report NOR-X319*. Section 14(ii). Forestry Canada Northwest Region, Northern Forestry Centre, Edmonton, Alberta

23.4 Organic (LFH) Soil Sample Analysis

A field-moist sample is required for this analysis (**SOIL SAMPLE PREPARATION PROCEDURE (#9)**). An amount approximately equivalent to 2 g dry weight of LFH material is to be extracted and analysed. **SOIL SAMPLE PREPARATION PROCEDURE (#9)** describes the method by which the moisture content is to be determined, for calculation of inorganic sulphur concentration on a dry weight basis.

1. Blend field moist sample to uniform state.
2. Weigh a field-moist sample approximately equivalent to 2 g (weighed to ±0.01 g) into a polypropylene tube.
3. Add 20 mL 0.01 *M* NH_4Cl.
4. Shake for 1 hr.
5. Centrifuge 2,000g for 10min.
6. Filter through 0.45 µm nylon membrane syringe filter.
7. If analysis cannot take place within 24 hr after extraction, freeze (-20°C) clarified samples until analysis can be completed.
8. Use ICP-AES to determine total sulphur concentration in an aliquot of the extract.
9. Use ion chromatography to determine sulphate (SO_4^--S) concentration in an aliquot of the extract.

23.5 Mineral Soil Sample Analysis

An air-dried sample, passed through a 2 mm screen, is required for this analysis.

1. Weigh 2 g (±0.01 g) of 2 mm air dry mineral soil.
2. Add 20 mL $Ca(H_2PO_4)_2$ solution.
3. Shake for 1 hr.
4. Centrifuge 2,000g for 10min.
5. Filter through 0.45 um nylon membrane syringe filter.
6. If analysis cannot take place within 24 hr after extraction, freeze (-20°C) clarified samples until analysis can be completed.
7. Use ICP-AES to determine total sulphur concentration in an aliquot of the extract.

23.6 Calculations

Report total dissolved sulphur and sulphate-S concentrations in both LFH and mineral soils on a dry weight basis.

PROCEDURE #24
TREE MAPPING MEASUREMENTS

24.1 Background 55
24.2 Vegetation Plot Tree Mapping Measurements.....55
24.3 Edge Plot Tree Mapping Measurements 56

24.1 Background

The location of each vegetation plot tree is to be measured relative to the plot centre. These measurements will be used to prepare a map of trees within the plot.

A map of the edge monitoring plot is also to be prepared. Measurements are to be taken from the corner of the plot closest to the reference stake.

24.2 Vegetation Plot Tree Mapping Measurements

Each tree within the vegetation plot is to be mapped as a function of distance from the plot centre. Measurements (±0.1 m) are to be recorded on TEEM Form 02. Open fields in the form are to be completed as described below.

TEEM Form 02 – Vegetation Plot Tree Map

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the map information from the last plot tree. If vegetation plot map data are collected over a two or more days, a new form is to be used at the start of each day. The total number of pages used to collect the plot map data is to be entered into Field 2
3 to 7	Site	5-Character site designation
8 to 11	Year of Establishment	The 4-digit year of plot establishment
12 to 19	Assessment Date	The date of vegetation plot map data collection, in the format YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
20 to 22	Personnel	The full names of field personnel collecting the plot map data
23 to 25	Tree Number	A 3-digit number, such that the 26th mapped tree would be recorded as “026”
28	Status	“A“= alive and “D” = dead
29 to 32	Distance Along Centre Line (X)	The centre of each tree bole, measured from the plot centre (“0,0”). Plus (+) and minus (-) signs (Field 29) designate the plot quadrant. Distances along the X axis are measured from 0 to 5.0 m (±0.1 m)
33 to 37	Distance Along Reference Line (Y)	The centre of each tree bole, measured from the plot centre (“0,0”). Plus (+) and minus (-) signs (Filed 29) designate the plot quadrant. Distances along the Reference Line (Y axis) are measured from 0 to 20.0 m (±0.1 m)
38	Remarks	Note any features of the tree that may be related to or affect the health of the tree, and any unusual observations

24.3 Edge Plot Tree Mapping Measurements

Each tree within the edge plot is to be mapped as a function of distance from the plot corner closest to the reference stake. Measurements (±0.1 m) along the long and short plot edges are to be recorded on TEEM Form E02. Open fields in the form are to be completed as described below.

TEEM Form E02 – Edge Monitoring Plot Tree Map

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the map information from the last plot tree. If vegetation plot map data are collected over a two or more days, a new form is to be used at the start of each day. The total number of pages used to collect the plot map data is to be entered into Field 2
3 to 7	Site	5-Character site designation
8 to 11	Year of Establishment	The 4-digit year of plot establishment
12 to 19	Assessment Date	The date of vegetation plot map data collection, in the format YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
20 to 22	Personnel	The full names of field personnel collecting the plot map data
23 to 25	Tree Number	A 3-digit number, such that the 7th mapped tree would be recorded as “E07”
28	Status	“A” = alive and “D” = dead
29 to 32	Distance Along Plot Long Edge	The centre of each tree bole, measured along the long edge of the plot from the plot corner closest to the reference stake. Distances are measured from 0 to 20 m, to tenths of metres.
33 to 35	Distance Along Plot Short Edge	The centre of each tree bole, measured along the short edge of the plot, from the plot corner closest to the reference stake. Distances are measured from 0 to 5 m, to tenths of metres.
36	Remarks	Note any features of the tree that may be related to or affect the health of the tree, and any unusual observations

PROCEDURE #25
TREE CORING

25.1 Background 57
25.2 Tree Coring 57

25.1 Background

The effects of air emissions and deposition on the growth and health of jack pine trees are expected to be observable on trees 50 to 70 years of age. The coring of trees provides a sample for analysis confirmatory of tree age estimated during the evaluation of the site.

25.2 Tree Coring

At breast height (1.3 m), a core through the pith of each numbered off-plot tree is to be obtained using an increment borer (Grissino-Mayer, 2003[20]; Maeglin, 1979[21]). A wide-diameter (5 mm) "Suunto" brand borer is recommended.

In the event that a core does not intersect the pith of the tree, a second core may be obtained from the same tree. If the pith is missed on the second attempt, the tree is to be removed from the program, a replacement tree selected according to the process described in Sections 9.2.1 and 9.2.2, and a core obtained from this tree.

The hole(s) in the tree trunk is(are) to be left open, as plugging may increase the potential for physical and/or pathogenic damage (Grissino-Mayer, 2003[20]).

Each core is to be stored in a plastic straw, which is to be stapled closed and affixed with a label prepared according to the **SAMPLE LABELLING PROCEDURE (#1)**. Small slits should be cut (lengthwise) into the straw to allow moisture to escape. Straws containing the cores are to be placed into in a PVC tube of 10 cm diameter and 25 cm length (capped) for transport, to minimize breakage. Cores are not to be frozen at any time.

[20] Grissino-Mayer HD (2003) A manual and tutorial for the proper use of an increment borer. Tree-Ring Res. 59:63-79.

[21] Maeglin RR (1979) *Increment Cores. How To Collect, Handle, and Use Them.* General Technical Report FPL 25, Forest Products Laboratory, Forest Service, United States Department of Agriculture. 18 pp.

PROCEDURE #26
TREE CORE PREPARATION & ANALYSIS

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26.1 Background

Tree cores are to be dried, securely mounted, and sanded to properly prepare the cores for analysis. Analysis is to be conducted using high-resolution digital scanning.

26.2 Core Drying

As soon as possible after return from the field, the cores are to be dried in their straws at 60°C, for a minimum of 5 days.

26.3 Mounting Cores

Mounting boards that can accommodate up to five cores are to be prepared. Five grooves each measuring 6 mm wide and 4 mm deep are to be cut into each board, with the grooves equally spaced.

1. Cut the end off of each straw, and push the core out of the straw with a piece of doweling. If the core is in pieces make sure that the orientation of each piece remains correct.
2. Place a thick bead of glue ("Lepage Sure Grip" carpenter's glue or equivalent) in one groove – the glue should come up the sides of the core but not spill onto the top of it. All pieces are to be placed in the board so that the vessels are aligned vertically in the mount (if the core is mounted out of phase the rings will not be visible, and the sample will be unusable). Any shiny areas on the core are to be orientated to the sides of the board.
3. If the core is twisted (i.e., starts in phase and then turns out of phase down the core), steam the core for about 1 minute on a kettle and gently twist the core straight. Place the core in the mount. If the cores start to curl, gently push them back into place.
4. Cover the cores using a small piece of plastic, and place a heavy object evenly over the mount.
5. Allow the glue to dry for 24 hours. Do not clamp the boards together. The cores are to be checked after 24 hours to confirm that they are securely mounted; if not, add more glue.

6. Sample label information for each of the mounted cores is to be written on the back of the board as any information on the front of board may be sanded off.

26.4 Sanding Cores

1. Each core is to be sanded first with 120 grit sandpaper to create a flat surface on the top of the mounted core. Sand the sample evenly, and avoid creating waves on the core surface.
2. Sand the flattened surface with 220 grit sandpaper; this may be done with a belt sander.
3. Sand the flattened surface with 320 grit sandpaper; this may be done with a belt sander.
4. Sand the flattened surface with 500 grit sandpaper; this must be done by hand, with the sandpaper installed into a hand sander that is clamped into a vice. The block and core are then to be pushed over the surface of the sandpaper.
5. Polish the flattened surface with 1,000 grit sandpaper; this must be done by hand with the sandpaper installed into a hand sander that is clamped into a vice. The block and core are then to be pushed over the surface of the sandpaper.

26.5 Scanning Cores

The highest resolution scanner available ("Epson Perfection V7500 Pro" or equivalent) is to be used to obtain electronic core images.

1. Place the core board perpendicular to the scanner bar.
2. Set the scanner to 1,800 DPI, or the highest resolution that it will scan before it extrapolates the resolution.
3. Scan a colour image to a bitmap (.bmp) file format (do not use .jpg). Limit the scan to the core (not the entire board) to minimize file size.
4. Carefully examine image for quality. If inadequate, additional sanding and/or scanning will be required.

26.6 Measuring Growth Rings

The ring measurement and cross dating programs "CooRecorder" and "C-Dendro"[22], respectively, are to be used to measure growth rings on each core.

1. Open the image of a core in "CooRecorder" and set the image DPI, and select "sorted data".
2. Begin measurement at the ring closest to the bark and then sequentially measure rings inward.

[22] CooRecorder and C-Dendro are available from Cybis Elektronik & Data AB, Pålnäsvägen 1, SE-133 33 Saltsjöbaden, Sweden (http://www.cybis.se/forfun/dendro/index.htm)

3. Measure straight across each ring, avoiding measurement at any angle.
4. The completed series is then to be brought into "C-Dendro" for validation and cross-dating and for converting data into ring width files.

The "C-Dendro" results are to be entered on TEEM Form X05. The number of growth rings, plus 10, in each core represents the age of the tree, providing that the core intersected the pith of the tree. The addition of 10 to the number of growth rings accounts for the number of years required for the tree to grow to breast height (1.3 m). The open fields in TEEM Form X05 are to be filled in as follows:

TEEM Form X05 – Off-Plot Tree Growth Ring Analysis

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete as appropriate. The first page (Fields 1 to 86) is identified by an "A"; the second page (Fields 87 to 132) is identified by a "B". If tree growth ring data are collected on different days, complete a separate form for each day. The total number of pages used to collect the growth ring data is to be entered into Field 2
3 to 7	Site	5-Character site designation
8 to 15	Tree Core Sample Date	The date tree cores are collected, in the format YYYY-MM-DD (July 9, 2011 would be recorded as "2011-07-09")
16 to 23	Tree Core Measurement Date	The date tree core ages are measured, in the format YYYY-MM-DD (July 9, 2011 would be recorded as "2011-07-09")
24 to 26	Cores Acquired By	Three fields are provided for the full name(s) of the personnel taking the tree core samples
27 & 28	Cores Measured By	Two fields are provided for the full name(s) of the personnel measuring the tree core samples
29 to 38	Off-Plot Tree Numbers	The 3-character tree number, in the form "X10"
40 to 129	Tree Ring Width by Year	The width of the current year tree ring is to be recorded (in mm, to 0.01 mm). Measurements are to be in mm, to 0.01 mm; 1.27 mm would be recorded as "127". The width of each ring working from the current year ring to the pith is to be recorded for as many rings as are present in the core
131	Tree age	The age of the tree is to be determined by counting the number of growth rings, and adding 10
132	Remarks	Observations of unusual growth years (e.g., fire scars), if a tree core did not intersect the pith and any correction applied to derive tree age, and any measurement difficulties (broken cores) are to be recorded

PROCEDURE #27
TREE CONDITION & HEALTH ASSESSMENT

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27.1 Background

Assessments of the health of the vegetation plot trees, the off-plot trees, and the edge plot trees are required annually. This assessment provides data and information that may assist in the interpretation of data collected at longer intervals (3 to 6 years).

27.2 Assessment – TEEM Forms 04, X04 & E04

Use of binoculars is required in order to see the tree crowns in adequate detail.

The same assessment procedure applies to trees in the vegetation plot, off-plot trees, and trees in the edge monitoring plot. The open fields in TEEM Form 04 (vegetation plot trees), TEEM Form X04 (off-plot trees), and TEEM Form E04 (edge plot trees) are to be completed as follows:

TEEM Forms 04 (Vegetation Plot), X04 (Off-Plot Trees) & E04 (Edge Plot Trees) – Annual Forest Health & Condition Assessment

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the data from the last plot tree
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	The date, in the format YYYY-MM-DD (July 9, 2011 would be recorded as 2011-07-09)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19 to 21	Tree Number	A 3-digit entry for each vegetation plot tree (tree number 54 would be recorded as “054”), or a 2-digit entry preceded by an “X” (off-plot tree, i.e., “X09”) or “E” (edge plot tree, i.e., “E07”)

Field(s)	Field Name	Required Information
25 & 26	Crown Condition (Defoliation)	Damage is defined as parts of the crown impacted by agents to the extent that the vigour of the tree is reduced. Numbered dead trees are to be recorded only so far as tree number, species, and type, with a straight line drawn through the remaining data fields with a "tree dead" note made in the Remarks field. Defoliation is any loss of needles beyond a level normal for the species. Overall conifer crown condition is recorded using the 2-digit codes: 01 = no defoliation; 02 = only current foliage defoliated, defoliation <25% 03 = current and/or some older foliage defoliated, defoliation <25%; 04 = 25 to 50% defoliation; 05 = 51 to 75% defoliation; 06 = 76 to 90% defoliation 07 = >90% defoliation; 09 = dead tree. Note any evidence of cause of death (blow-down, root rot, etc.)
27 to 29	Extent of Foliage Missing	The Extent of Foliage Missing codes (below) must be entered for each of the upper (U), middle (M), and lower (L) third of the crown. Note the symptoms of damage (dead twigs, branches and/or tops, missing foliage, disease, mistletoe, witches'-broom, etc.) in the Remarks field
30 & 31	Bare Top Length	The length of a bare top (dead branches above the live canopy) is to be estimated to the nearest 0.5 m, and entered as a two-digit number, with the estimated fraction (nearest 0.5 m) entered into the shaded cell. If the top of the tree cannot be seen, draw a line through these fields
32	Needle Retention	Assess needle retention in the middle and upper crown only. Needle retention is defined as the age of the oldest internode with at least 25% of the needles present. The age of the oldest shoots supporting at least 25% foliage is to be recorded according to the following codes: 0 = current year's foliage only; 1 = 1 year old; 2 = 2 years old; 3 = 3 years old; 4 = 4 years old; 5 = 5 years old and older - = unable to see into middle or upper crown
33	Storm Damage	Define the physical damage to the living main stem or damage to branches or twigs that constitutes at least 5% of the tree's live crown, excluding any dead areas of the crown due to other causes according to the following codes: 0 = No damage; 1 = foliage component damaged; 2 = live twigs broken; 3 = live branch(es) broken, other than main branch(es); 4 = live main branch(es) broken or severely cracked; 5 = live portion of main stem broken or severely cracked; 8 = Other (describe in remarks field); 9 = Not applicable, tree is dead
34	Current Foliage Missing	Examine the current year shoots, and visually estimate the amount of foliage missing (regardless of cause) at several locations in the live crown, using the Extent of Foliage Missing codes below

Field(s)	Field Name	Required Information
35 & 36 37 & 38	Abiotic Foliage Symptoms	These fields are reserved for recording discolouration and other symptoms of unknown (defined here as "abiotic") origin. If the cause of the symptom is identifiable, enter "00" and proceed to the next assessment. Record one or two conditions (note the suspected causal agent in Remarks field) using the following codes: 00 = green (normal); no undetermined abiotic symptoms 85 = chlorotic 86 = yellow current year needles 87 = chlorotic yellow old foliage 88 = mottled yellow, brown or red spots 89 = marginal tip or edge discolouration (red, brown, yellow) 90 = needle banding of all needles per fascicle 91 = needle banding of some needles per fascicle 92 = spotted, dead areas on needles 93 = interveinal discolouration (not used for conifers) 94 = entire needle red or brown 95 = needle tips dead and broken off at same length per fascicle 96 = needles on entire shoot or branch red or brown 99 = other, describe in Remarks
39	Extent of Foliage Affected	Record the extent of foliage affected by observed abiotic disorders, using the Extent of Damage codes below
40	Seed (cone) Production	Estimate and record using the following codes the amount of seed (cone) production for dominant and co-dominant trees only: 0 = no cones or seeds 1 = very light 2 = light 3 = medium 4 = heavy - = not applicable (e.g., tree is not dominant or co-dominant)
41	Type (T) of Foliar Insects	Identify and record the most common insect type present (if two are equally common, record the insect type causing the greater damage) using the following codes: 0 = none 1 = free-feeding defoliator (spruce budworm, forest tent caterpillar, etc.) 2 = leaf miner, needleminer (e.g., pine needleminer); 3 = tent or nestmaker (e.g., eastern tent caterpillar, spruce web-spinning sawfly) 4 = budminer, shootminer 5 - gallmaker 6 = sucking insects (aphids) 8 = other, specify in Remarks field **Non-Destructive Observations Only**
42	Extent (E) of Foliar Damage Caused by Insects	The level of insect damage in total (all damage regardless of species causing greatest damage) is to be recorded using the Extent of Damage codes below. **Non-Destructive Observations Only**

Field(s)	Field Name	Required Information
43	Type (T) of Foliar Disease	Identify and record the most common disease present (if two are equally common, record the disease causing the greater damage) using the following codes: 0 = none 1 = needle rust 2 = needle cast 3 = needle blight 4 = leaf spot (not used for conifer) 5 - anthracose 6 = leaf blisters (not used for conifer) 7 = physical damage (wind, hail, frost) 8 = other, specify in Remarks
44	Extent (E) of Foliar Damage Caused by Diseases	The level of disease damage in total (all damage regardless of species causing greatest damage) is to be recorded using the Extent of Damage codes below.
45	Type (T) of Woody Tissue Insect	Identify and record the insect most likely to affect the health of the tree (e.g., an insect likely to kill the tree would be recorded, not the insect likely to cause only needle damage, if both were present) using the following codes: 0 = none 1 = sucking insect 2 = gall maker 3 = tip borer 4 = bark beetles 5 - wood boring insects 6 = root collar insects 8 = other, specify in Remarks field
46	Location (L) of Woody Tissue Insect Damage	Record the location of damage caused by the type of insect recorded in field 50 using the Damage Location codes below.
47	Type (T) of Woody Tissue Disease	Identify and record the disease most likely to affect the health of the tree (e.g., a disease likely to kill the tree would be recorded, not the disease likely to cause only needle damage, if both were present) using the following codes: 0 = none 1 = stem and branch rust 2 = stem canker 4 = twig or branch canker 5 - witch's broom, mistletoe 6 = stem decay fungi 7 = root rot 8 = other, specify in Remarks
48	Location (L) of Woody Tissue Disease Damage	Record the location of damage caused by the type of disease recorded in field 47 using the Damage Location codes below

Field(s)	Field Name	Required Information
49	Type (T) of Other Damaging Agent	The type of any other damage not yet recorded that is likely to affect the health of the tree is to be recorded (if more than one is present, record the one most likely to affect tree health), as follows: 0 = no visible damage 1 = animal (mammal, bird) 2 = snow 3 = ice 4 = hail 5 - wind 6 = frost 7 = mechanical (whipping, etc.) 8 = other, specify in Remarks
50	Location (L) of Damage Caused by Other Agents:	Record the location of damage caused by the type of insect recorded in field 49 using the Damage Location codes below
51	Extent (E) of Woody Tissue Damage Caused by Other Agents	The level of woody tissue damage in total (all damage regardless of cause of greatest damage) is to be recorded using the Extent of Damage codes below
52	Remarks	In addition to the requirements above to note particular observations in the Remarks, any observations that would assist in the interpretation of data gathered during plot establishment, or may provide insight into tree health and death during data collection during the routine monitoring program should be entered in this field

27.3 Extent of Foliage Missing & Current Foliage Missing Codes for TEEM Forms 04, X04 & E04

The Extent of Foliage Missing codes (below) are to be entered for each of the upper (U), middle (M), and lower (L) third of the crown. Note the symptoms of damage (dead twigs, branches and/or tops, missing foliage, disease, mistletoe, witches'-broom, etc.) in the Remarks (Field 52). Examine the current year shoots, and visually estimate the amount of foliage missing (regardless of cause) at several locations in the live crown, using the Extent of Foliage Missing codes, below:

Code	Extent of Foliage Missing (Fields 27 to 29, 34)	Extent of Damage (Fields 39, 42, 44 & 51)
0	None	None
1	1 to 25%	1 to 25%
2	26 to 50%	26 to 50%
3	51 to 75%	51 to 75%
4	76 to 100%	76 to 100%
-	Not Applicable	Not Applicable
X	Cannot see section of crown, unable to rate	Cannot see section of crown, unable to rate

27.4 Location of Woody Tissue Insect Damage, Disease and Other Damaging Agent Codes for TEEM Forms 04, X04 & E04

Record the location of damage caused by the type of insect recorded in field 45 using the Damage Location codes below.

Code	Location of Woody Tissue Insect Damage (Field 46), Disease (Field 48) and Other Damaging Agent (Field 50)
0	No visible damage
1	Crown stem (main trunk within the crown)
2	Upper bole (upper half of the trunk between the roots and the base of the crown)
3	Lower bole (lower half of the trunk between the roots and the base of the crown)
4	Roots (exposed) and stump (up to 25 cm in height)
5	Whole trunk (includes trunk evaluated in codes 1 to 3)
6	Branches and twigs, the woody stems other than main stem
7	Shoots and buds, considering current year's growth only
8	Whole crown (includes crown components evaluated in codes 6 and 7)
9	Other, specify in Remarks field

In addition to the requirements above to note particular observations in the Remarks, any observations that would assist in the interpretation of data gathered during plot establishment, or may provide insight into tree health and death during data collection during the routine monitoring program should be entered in this field.

PROCEDURE #28
TREE DATA

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28.1 Background

Tree data are required from each numbered and mapped tree within the vegetation plot, the off-plot trees, and the numbered edge monitoring plot trees during each cycle of monitoring. Tree measurements and observations are to be obtained as described below, with the observations recorded in TEEM Form 03 (vegetation plot trees), X03 (off-plot trees) or E03 (edge plot trees), as appropriate.

During the measurement process, tree tags are and DBH reference marks are to be checked and as required, repaired or replaced (**TREE NUMBERING & LABELLING PROCEDURE (#5)**).

28.2 Common Procedures

DBH and height measurements follow the procedures immediately below. The recording of morphological observations is described in association with the TEEM Data Forms (03, X03, E03), in subsequent sections (below).

28.2.1 Dominance (Field 24)

Dominance of each numbered tree is coded as follows:

1 = Dominant – the tree crown extends above the general level of the crown canopy, and receives full sunlight from above and partial sunlight from the sides.

2 = Co-dominant – the tree crown is at the general level of the crown canopy, receiving full sunlight from above but little sunlight from the sides.

3 = Intermediate – tree crown extends into the general level of the crown, but is shorter than co-dominant trees, receiving little direct sunlight from above.

4 = Suppressed – tree crown entirely below the general level of the canopy.

The dominance of dead trees is to be estimated at the time when they were last alive

28.2.2 Diameter at Breast Height (Fields 25 to 28)

The diameter of the tree at breast height (DBH) is to be measured at the line painted on the trunk 1.3 m above ground level (**TREE NUMBERING & LABELLING PROCEDURE (#5)**) using a diameter tape, the measurement taken to the nearest 0.1 cm and recorded as a four-digit number (e.g., a DBH of 10.3 cm is recorded as 0103). Abnormalities in tree form (forked stems, branch or swelling at 1.3 m) are to be noted, and adjustments in the diameter measurement (at higher or lower than 1.3 m) are to be recorded.

28.2.3 Crown Closure (Field 29)

For each living dominant and co-dominant tree, an estimate of the number of sides of the tree's crown that touch or overlap with neighbouring dominant and co-dominant trees is to be made according to the following codes:

0 = Tree does not touch or overlap any neighbouring Dominant or Co-dominant tree.

1 = Neighbouring trees touch or overlap one quadrant of the subject tree.

2 = Neighbouring trees touch or overlap two quadrants of the subject tree.

3 = Neighbouring trees touch or overlap three quadrants of the subject tree.

4 = Neighbouring trees touch or overlap four quadrants of the subject tree.

8 = Not applicable, subject tree is intermediate or suppressed.

28.2.4 Stem Form (Field 30)

Taking into account the normal stem form for jack pine, record stem form according to the following codes:

0 = Normal stem, no abnormalities.

1 = Main stem broken off.

2 = Top of tree broken off.

3 = Main stem abnormally forked below the living crown.

4 = Stem significantly twisted (into a spiral).

5 = Tree leaning more than 15° from vertical.

9 = Other (describe in remarks field).

- = Not applicable, tree is dead.

28.2.5 Tree Height (Fields 31 to 33) and Crown Heights (Fields 34 to 39)

Total height, the height to the top of the tree (fields 31 to 33), is to be measured using a laser rangefinder. The measurement is taken to the nearest 0.1 m, and recorded on the data sheet as a three-digit number (e.g., 18.9 m is to be recorded as “189”).

Height to the top of the live crown (fields 34 to 36) is to be measured in the instance where dead branches form the top of the tree. If the top of the tree is alive, the Total Height may be recorded as the Height to the Top of Live Crown without repeating the measurement. The measurement is taken to the nearest 0.1 m, and recorded on the data sheet as a three-digit number (e.g., 18.1 m is to be recorded as “181”).

Height to the base of the crown (fields 37 to 39) is to be measured from the ground to the bottom of the living, productive foliage of the lowest branch. This is a subjective decision, based on the decision of whether the branch contributes to the health of the tree. The measurement is taken to the nearest 0.1 m using a laser rangefinder, and recorded on the data sheet as a three-digit number (e.g., 15.2 m is to be recorded as “152”).

28.2.6 Remarks (Field 40)

Tree characteristics that pose difficulties in any of the measurements should be noted, and any decisions made that will affect future measurements must be recorded (e.g., “DBH measured at a height of 1.6 m, 30 cm above abnormal swelling”).

Personnel are encouraged to include in the Remarks field any observation that may assist in data interpretation and/or the understanding of the status of tree, plot, or site health.

28.3 Vegetation Plot Tree Data

Tree data are required from each numbered and mapped tree within the vegetation plot; these data are to be recorded using TEEM Form 03; the open fields in TEEM Form 03 are to be completed as follows:

TEEM Form 03 – Vegetation Plot Tree Data

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the data from the last plot tree
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19 to 21	Tree Number	A 3-digit number, such that the 26th mapped tree would be recorded as “026”
24	Dominance	Dominance of each numbered tree is coded according to Section 21.2.1 (above)
25 to 28	DBH	The diameter of the tree at breast height is to be measured according to Section 21.2.2 (above)
29	Crown Closure	Crown closure described according to Section 21.2.3 (above)
30	Stem Form	Stem form described according to Section 21.2.4 (above)
31 to 33	Total Height	Total tree height measured according to Section 21.2.5 (above)
34 to 36	Height to Top of Live Crown	Height to top of live crown measured according to Section 21.2.5 (above)
37 to 39	Height to Base of Crown	Height to base of live crown measured according to Section 21.2.5 (above)
40	Remarks	Observations related to the status of tree, plot and/or site health status

28.4 Off-Plot Tree Data

Tree data are required from each numbered and mapped tree within the edge monitoring plot; these data are to be recorded using TEEM Form X03; the open fields in TEEM Form X03 are to be completed as follows:

TEEM Form X03 – Off-Plot Tree Data

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete as appropriate
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19 to 21	Tree Number	A 2-digit number, following the “X” that is pre-entered in Field 19 on the form, such that the 4th tree would be recorded as “X04”
24	Dominance	Dominance of each numbered tree is coded according to Section 21.2.1 (above)
25 to 28	DBH	The diameter of the tree at breast height is to be measured according to Section 21.2.2 (above)
29	Crown Closure	Crown closure described according to Section 21.2.3 (above)
30	Stem Form	Stem form described according to Section 21.2.4 (above)
31 to 33	Total Height	Total tree height measured according to Section 21.2.5 (above)
34 to 36	Height to Top of Live Crown	Height to top of live crown measured according to Section 21.2.5 (above)
37 to 39	Height to Base of Crown	Height to base of live crown measured according to Section 21.2.5 (above)
40	Remarks	Observations related to the status of tree, plot and/or site health status

28.5 Edge Monitoring Plot Tree Data

Tree data are required from each numbered and mapped tree within the edge monitoring plot; these data are to be recorded using TEEM Form E03; the open fields in TEEM Form E03 are to be completed as follows:

TEEM Form E03 – Edge Monitoring Site Tree Data

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the data from the last plot tree
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Assessed By	Three fields are provided for the full names of the personnel involved
19 to 21	Tree Number	A 2-digit number, following the “E” that is pre-entered in Field 19 on the form, such that the 4th tree would be recorded as “E04”
24	Dominance	Dominance of each numbered tree is coded according to Section 21.2.1 (above)

Field(s)	Field Name	Required Information
25 to 28	DBH	The diameter of the tree at breast height is to be measured according to Section 21.2.2 (above)
29	Crown Closure	Crown closure described according to Section 21.2.3 (above)
30	Stem Form	Stem form described according to Section 21.2.4 (above)
31 to 33	Total Height	Total tree height measured according to Section 21.2.5 (above)
34 to 36	Height to Top of Live Crown	Height to top of live crown measured according to Section 21.2.5 (above)
37 to 39	Height to Base of Crown	Height to base of live crown measured according to Section 21.2.5 (above)
40	Remarks	Observations related to the status of tree, plot and/or site health status

PROCEDURE #29
TREE SHOOT DATA

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29.1 Background

Internode growth measurements and estimates of the retention of needles on each internode are to be completed on the cut branch, prior to the acquisition of needle samples for laboratory analyses.

29.2 Photography

Prior to conducting any foliar assessment, measurement or sampling, each cut branch is to be photographed, and the photograph number is to be noted on TEEM Form X06 (below).

29.3 Internode Measurement

The length of each internode on the each of the five main branches is to be measured (in cm, to 0.1 cm) using a ruler or calipers. Measurements are to be recorded using three digits (e.g., 4.9 cm is to be recorded as “049”).

29.4 Needle Retention

Defoliation of each internode is to be estimated according to the codes, below. Measurements and defoliation estimations are to be carried back from the current year internode for at least 4 more years (to the 4-year-old age class), and to the 7-year-old age class if possible. Defoliation estimates are quantified as follows:

0 = None
1 = 1 to 25% defoliation
2 = 26 to 50% defoliation
3 = 51 to 75% defoliation
4 = 76 to 100% defoliation
- = Not applicable

These data and observations are to be recorded in the Defoliation fields (26, 30, 34, 38, 42, 46, 50 & 54) in TEEM Forms X06 (off-plot trees) and E06 (edge plot trees), below.

29.5 Off-Plot Tree Shoot Data

The open fields in TEEM Form X06 are to be completed as follows:

TEEM Form X06 – Off-Plot Tree Shoot Data

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete as appropriate
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	The date, in the format YYYY-MM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19 & 20	Random number	The random number used to select the five off-plot trees for sampling. Enter as a 2-digit number (e.g., random number “3” is to be entered as “03”)
21 to 23	Off-Plot Tree Numbers	A 2-digit number, following the “X” that is pre-entered on the form. The 10th tree would be recorded as “X10”
26	Current Year Internode Defoliation	The extent of defoliation in the current year internode
27 to 29	Current Year Internode Length	The length of the current year internode
30	1 Year Old Internode Defoliation	The extent of defoliation in the 1-year-old internode
31 to 33	1 Year Old Internode Length	The length of the 1-year-old internode
34	2 Year Old Internode Defoliation	The extent of defoliation in the 2-year-old internode
35 to 37	2 Year Old Internode Length	The length of the 2-year-old internode
38	3 Year Old Internode Defoliation	The extent of defoliation in the 3-year-old internode
39 to 41	3 Year Old Internode Length	The length of the 3-year-old internode
42	4 Year Old Internode Defoliation	The extent of defoliation in the 4-year-old internode

Field(s)	Field Name	Required Information
43 to 45	4 Year Old Internode Length	The length of the 4-year-old internode
46	5 Year Old Internode Defoliation	The extent of defoliation in the 5-year-old internode
47 to 49	5 Year Old Internode Length	The length of the 5-year-old internode
50	6 Year Old Internode Defoliation	The extent of defoliation in the 6-year-old internode
51 to 53	6 Year Old Internode Length	The length of the 6-year-old internode
54	7 Year Old Internode Defoliation	The extent of defoliation in the 7-year-old internode
55 to 57	7 Year Old Internode Length	The length of the 7-year-old internode
58	Remarks	Observations of interest unrelated to the internode measurements or defoliation estimates
59	Photo. No.	The branch photograph number

29.6 Edge Plot Tree Shoot Data

The open fields in TEEM Form E06 are to be completed as follows:

TEEM Form E06 – Edge Monitoring Site Tree Shoot Data

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete as appropriate
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	The date, in the format YYYY-MM-DD (July 9, 2011 would be recorded as "2011-07-09")
16 to 18	Assessed By	Three fields are provided for the full names of the personnel involved
19 & 20	Random Number	The random number used to select the five edge monitoring site trees for sampling. Enter as a 2-digit number (e.g., random number "3" is to be entered as "03")
21 to 23	Edge Plot Tree Number	A 2-digit number, following the "E" that is pre-entered on the form. The 12th tree would be recorded as "E12"
26	Current Year Internode Defoliation	The extent of defoliation in the current year internode
27 to 29	Current Year Internode Length	The length of the current year internode
30	1 Year Old Internode Defoliation	The extent of defoliation in the 1-year-old internode
31 to 33	1 Year Old Internode Length	The length of the 1-year-old internode
34	2 Year Old Internode Defoliation	The extent of defoliation in the 2-year-old internode
35 to 37	2 Year Old Internode Length	The length of the 2-year-old internode
38	3 Year Old Internode Defoliation	The extent of defoliation in the 3-year-old internode
39 to 41	3 Year Old Internode Length	The length of the 3-year-old internode
42	4 Year Old Internode Defoliation	The extent of defoliation in the 4-year-old internode
43 to 45	4 Year Old Internode Length	The length of the 4-year-old internode
46	5 Year Old Internode Defoliation	The extent of defoliation in the 5-year-old internode
47 to 49	5 Year Old Internode Length	The length of the 5-year-old internode

Field(s)	Field Name	Required Information
50	6 Year Old Internode Defoliation	The extent of defoliation in the 6-year-old internode
51 to 53	6 Year Old Internode Length	The length of the 6-year-old internode
54	7 Year Old Internode Defoliation	The extent of defoliation in the 7-year-old internode
55 to 57	7 Year Old Internode Length	The length of the 7-year-old internode
58	Remarks	Observations of interest
59	Photo. No.	The branch photograph number

PROCEDURE #30
FOLIAR SAMPLE (ROUTINE) COLLECTION & CHECKLIST

30.1 Background ……..…..78
30.2 Foliar Sample (Routine) Checklist – Off-Plot Trees……..……..…....78
30.3 Foliar Sample Checklist – Edge Plot Trees……..…79

30.1 Background

Needles from each of the current annual growth (CAG), 1-year-old (Age-1) and 2-year-old (Age-2) internodes are to be collected for laboratory analyses. The amount of sample collected must be sufficient to allow completion of the required laboratory analyses, with some material remaining should one or a few analyses need to be repeated.

Trees are to be sampled during routine monitoring cycles, every 6 years for off-plot trees and every 3 years for edge plot trees.

30.2 Foliar Sample (Routine) Checklist – Off-Plot Trees

Personnel are to wear powderless nitrile gloves when handling branches and needles. Clippers pre-washed with isopropyl alcohol are to be used to cut shoot segments into the required age classes.

Current annual growth (CAG) needles, 1-year-old needles (Age-1) and 2-year-old needles (Age-2) from cut off-plot tree branches are to be sampled separately. A sample that will yield 15 to 20 g of dry foliar material is to be obtained for each needles age class. A minimum sample is 10 g, on a dry weight basis. In the event that needle material is limited, a second branch may be obtained to obtain this minimum quantity.

From the five branches excised for needle sampling, one of the branches having sufficient needle material to allow the collection of a second sample set (CAG, Age-1, Age-2) is to be randomly chosen. To obtain a field duplicate sample, twice the number or volume of shoots (e.g., equivalent to 20 g dry weight) is to be obtained and placed on a clean surface. The shoots are to be gently mixed, taking care to not break needles. The mixed sample is to be divided into two equal portions, one as the sample from the off-plot tree, the other as the field duplicate for the site. A total of 18 samples to be obtained per site, per the checklist below:

Foliar Sample (Routine) Checklist – Off-Plot Trees

Age Class	Number of Off-Plot Trees	Total Number of Samples
CAG	5	15
Age-1	5	
Age-2	5	
Field Duplicate Set (CAG, Age-1, Age-2)	1	3
	Total Samples per Site	**18**

30.3 Foliar Sample Checklist – Edge Plot Trees

A total of 12 samples to be obtained per edge site during each routine (3-year) monitoring cycle, per the checklist below:

Foliar Sample (Routine) Checklist – Edge Plot Trees

Age Class	Number of Edge Plot Trees	Total Number of Samples
CAG	3	9
Age-1	3	
Age-2	3	
Field Duplicate Set (CAG, Age-1, Age-2)	1	3
	Total Samples per Site	**12**

PROCEDURE #31
LICHEN TISSUE SAMPLE COLLECTION

31.1 Background 80
31.2 Sample Collection 80

31.1 Background

Lichen samples are to be collected from trees that are outside of the vegetation and soil plots. The species to be sampled is(are) to be confirmed in discussion with the TEEM Program Manager.

31.2 Sample Collection

Three separate, replicate samples of approximately 2 g each of the selected lichen species (typically *Hypogymnia physodes* and *Evernia mesomorpha)* are to be collected into clean, plastic sealable bags. Lichens are to be collected from tree branches only, not tree boles, at a height of at least 1.5 m from the forest floor, since lichens growing at lower heights may be less exposed to atmospheric deposition (e.g., may be covered in snow during part of the year). Sampling is to ensure that:

- only live lichens are to be sampled; dusty, discoloured, or decaying lichen individuals are to be avoided;
- the whole of the lichen individual complete with the thallus that connects to the tree branch is collected; this is more difficult when the sample is dry;
- close attention is paid to contamination of gloves; if contamination of gloves is suspected, immediately discard them and put on new gloves;
- lichen individuals are placed into a sample bag immediately; do not collect a handful of lichen and transfer it in bulk to the sample bag;
- if necessary, a stainless steel knife may be used to aid in sample collection but must be cleaned and stored in soapy water or alcohol after and in between each site;
- as much non lichen debris (bark, twigs, insect remains, etc.) is to be removed before placing lichen tissues in the sample bag; and
- tissues are collected only from branches of standing (live or dead) trees above 1.5 m. Tissues are not to be taken from the bole of the tree, downed trees, or from the litter of forest floor.

A field duplicate sample of lichen material is to be obtained from 10% of the sites being sampled in a single year, rounded up to the next whole number (1 field duplicate for up to 10 sites, 2 field duplicate samples for 11 to 20 sites; 3 for 21 to 30 sites, etc.). A field duplicate is to be prepared

by acquiring twice the amount of lichen tissue required for a sample, mixing the material gently (minimizing breakage of individual lichens), and dividing the sample into two equal portions, one as the site sample, the second as the field duplicate.

Care must be taken to acquire only the minimum sample required, as excessive sampling may denude the site of the lichen, compromising the long-term program.

Each sample is to be placed into a labelled plastic zipper storage bag, which itself is to be placed into a second, labelled plastic zipper storage bag. Lichen samples are to be placed in a cooler with sufficient ice packs to maintain a constant, cool sample temperature. Upon return from the field, samples are to be transferred to properly labelled brown paper bags. This transfer must occur on the same day as sampling. The **SAMPLE LABELLING PROCEDURE (#1)** and **SAMPLE STORAGE & SHIPPING PROCEDURE (#2)** both apply to the handling of lichen samples.

PROCEDURE #32
TREE & LICHEN TISSUE SAMPLE PREPARATION

32.1 Background 82
32.2 Sample Drying 82
32.3 Sample Cleaning 82
32.4 Sample Grinding.....83
32.5 Sample Re-drying.....83
32.6 Sample Weighing.....83
32.7 Sample Archive 83

32.1 Background

This procedure describes the handling and preparation of needle, bark and lichen samples for laboratory analyses. Handling of these samples is generally to be conducted wearing nitrile gloves, however, removal of fascicle material is difficult requiring dexterity not possible while wearing gloves. In this instance, careful handling of the sample without gloves is permitted.

32.2 Sample Drying

Drying of samples results in the cessation of cellular activity, both in tissue and microbial cells. This is a requirement of sample preservation. It is also necessary to dry needle samples to facilitate fascicle removal and to ensure proper grinding.

Samples are to be dried in small, labelled (**SAMPLE LABELLING PROCEDURE (#1)**) paper bags in an oven maintained at 70°C for 24 hr (needles, bark) or for 72 hr (lichen).

32.3 Sample Cleaning

On a clean, inert surface, needles are to be carefully removed from the branch segments, and the fascicles removed. Bark samples are to be inspected and extraneous material (e.g., lichen) is to be carefully removed. Lichen samples are to be cleaned of any residual bark, insect or other foreign materials.

The samples are to be immediately ground (see below). If grinding is delayed, the samples are to be placed in a glass vial, capped and stored in cool, dry conditions until drying capacity becomes available. Alternatively, dried samples can be placed into labelled paper bags, and sealed in a larger plastic bag, until grinding becomes possible.

32.4 Sample Grinding

Grinding reduces the material to small particles, which permits complete mixing and analyses of a homogeneous subsample representative of the larger sample.

Within 1 hour of removal from the oven, each dried sample is to be ground in zirconium oxide jar sets. The ground material is to be transferred into a cleaned glass vial, and sealed completely to prevent sample exposure to atmospheric moisture. The dried, ground sample is to fill the container to no more than two-thirds capacity, leaving sufficient head-space required to re-mix the sample by rolling, tilting and inverting immediately prior to removing a subsample for analysis.

32.5 Sample Re-drying

Dried, ground samples are to be re-dried immediately prior to the weighing of a subsample for each analysis. Loosely capped vials containing ground materials are to be placed in an oven (70 °C) overnight, and the caps securely tightened the following morning.

After the vial has cooled to room temperature, a subsample can be removed and weighed for the laboratory analysis. Providing that the vials remain securely sealed, the re-drying process does not need to be repeated daily. If the samples have been stored for an extended period (a month or more), re-drying is necessary.

32.6 Sample Weighing

1. Gently roll and tilt the glass jar containing the dried, ground sample for 10 sec to ensure complete mixing of the ground material.
2. Allow the mixed sample to sit until suspended particles have settled.
3. Remove and weigh the appropriate amount of sample for analysis (according to the individual procedure).
4. Securely seal the sample container immediately after weighing.

After weighing, sealed sample containers are to be stored in cool, dark conditions.

32.7 Sample Archive

Any remaining sample is to be placed into the TEEM sample archive facility. Samples are to be stored in tightly capped, labelled (**SAMPLE LABELLING PROCEDURE (#1)**), glass vials.

PROCEDURE #33
FOLIAR TISSUE INORGANIC SULPHUR ANALYSIS

33.1 Background **84**
33.2 Reagents: **84**
33.3 QA/QC **84**
33.4 Extraction & Analysis **84**
33.5 Calculations **85**

33.1 Background

The weak acid digestion procedure for sulfate in plant material is based on Brockley (2000[23]), followed by ion chromatography.

33.2 Reagents:

26 Water of ASTM Type I quality

27 0.01 *M* HCL

33.3 QA/QC

1. Standard solutions for calibration of the analytical instrument.
2. Reference standard (1 per batch): 0.5 g (±0.01 g) of dried, ground needles of known inorganic sulphur content
3. Blank (1 per batch): a digestion tube without sample.
4. Replicate (1 per batch): from one randomly selected sample within the batch.

33.4 Extraction & Analysis

1. Weigh 0.5 g (±0.01 g) of dried, ground needles into a pre-weighed digestion tube.
2. Add 40 mL 0.01 *M* HCl.
3. Boil for 1 hr.
4. Weigh tube with sample, calculate loss of water.
5. Pour aliquot into 15 mL centrifuge tubes, centrifuge at 2,000 g for 15 min
6. Filter extract through 0.45 µm Millipore (or equivalent) filter.
7. Analyse an aliquot of the extract using ion chromatography.

[23] Brockley RP (2000) Using foliar variables to predict the response of lodgepole pine to nitrogen and sulphur fertilization. Can. J. For. Res. 30:1389-1399

33.5 Calculations

The concentration of inorganic sulphur (S_i) in the sample is to be expressed in μg/g, on a dry-weight (moisture-corrected), water-loss basis.

PROCEDURE #34
FOLIAR TISSUE ORGANIC SULPHUR (SO) & SI:SO CALCULATIONS

34.1 Background 86
34.2 Organic Sulphur Concentration 86
34.3 Si:So Ratio Calculation 86

34.1 Background

Organic sulphur in foliar samples is determined by a calculation using the results of the total sulphur and inorganic sulphur analyses. The S_i:S_o ratio is also derived by calculation.

34.2 Organic Sulphur Concentration

The concentration of organic sulphur (S_o) in each foliar (needle) sample is derived through the subtraction of the foliar inorganic sulphur (S_i) concentration (**FOLIAR TISSUE INORGANIC SULPHUR ANALYSIS (#33)**) from the total foliar sulphur (S_t) concentration (**TOTAL SULPHUR, NITROGEN & CARBON ANALYSIS PROCEDURE (#17)**):

$$S_o = S_t - S_i$$

S_o is to be recorded in units of μg S_o/g DW.

34.3 Si:So Ratio Calculation

The S_i:S_o ratio is derived by dividing the concentration of foliar inorganic sulphur (S_i) (**FOLIAR TISSUE INORGANIC SULPHUR ANALYSIS (#33)**) by the concentration of foliar organic sulphur, derived above:

$$S_i:S_o = S_i/S_o$$

This ratio is unitless.

PROCEDURE #35
TREE TISSUE ELEMENTAL CONCENTRATIONS ANALYSIS

35.1 Background 87
35.2 QA/QC 88
35.3 Reagents 88
35.4 Microwave Digestion 88
35.5 Calculations 89

35.1 Background

This procedure is may be used to analyse for levels of a large number of elements in tree tissues. Of primary interest are the elements listed in the table below. Nevertheless, analysis using FLAA, CVAA, GFAA, ICP- AES, or ICP-MS allows sensitive, simultaneous determination of a broader suite of elements. Concentrations of elements not included in the following table are to be reported, however, for data management reasons, these results are to be captured in a secondary elements database, which is archived for potential future use.

Some element results are trustworthy only if specialized sampling and sample handling procedures were employed during sample acquisition (e,g,, As, Hg, Pb, Se).

Elements to be Included in the Priority Elements Database

Element		Emitted in Region	Nutrient	Toxic*
Aluminum	Al	Yes	No	Yes
Calcium	Ca	Yes	Macronutrient	No
Copper	Cu	Yes	Micronutrient	No*
Iron	Fe	Yes	Micronutrient	No*
Magnesium	Mg	Maybe	Micronutrient	No*
Manganese	Mn	Yes	Micronutrient	No*
Molybdenum	Mo	Yes	Micronutrient	No*
Nickel	Ni	Yes	No	Yes
Phosphorus	P	No	Macronutrient	No
Potassium	K	Yes	Macronutrient	No
Sodium	Na	Yes	(Micronutrient?)	No
Sulphur	S	Yes	Macronutrient	No*
Zinc	Zn	Yes	Micronutrient	No*

* Indicates no toxicity to vegetation at nutrient levels, toxicity at higher levels.

35.2 QA/QC

1. A set of calibration standards appropriate for the instrument.
2. Appropriate commercial standards, matching the sample matrix as closely as possible, or use of spiked samples to assess instrument response.
3. Reference standard (1 per batch): an appropriate amount of tree tissue material, of known total content of one or more elements.
4. Replicate (1 per batch): from one randomly selected sample within the batch.

35.3 Reagents

1. Water of ASTM Type I quality.
2. Concentrated HNO_3.
3. Concentrated HCl.

35.4 Microwave Digestion

Control of digestion conditions requires a temperature sensor in one or more vessels during the entire decomposition. The microwave decomposition system should sense the temperature to within ±2.5°C and permit adjustment of the microwave output power within 2 sec. Temperature sensors should be accurate to ±2°C (including the final reaction temperature of 180°C). The procedure requires microwave-transparent, reagent-resistant, and suitably inert reaction vessels. All sample containers must be prewashed with detergents, acids, and water.

1. Weigh 0.25 g (±0.001 g) of dried, ground sample into a digestion vessel.
2. Add 9 mL (±0.1 mL) concentrated HNO_3 and swirl the vessel gently so that all material comes in contact with the acid.
3. Digest according to microwave specifications.
4. Add HCl to stabilize elements in solution, concentration & volume to match matrix of the calibration standards.
5. Transfer and bring to desired volume (50 mL or 100 mL), using a diluent that matches the solvent used to prepare the standards.
6. If the digested sample contains particulates, centrifuge (up to 3,000 rpm, 10 min.) or allow particulates to settle (overnight).
7. Analyse solution elemental concentrations using flame atomic absorption spectrometry (FLAA), graphite furnace atomic absorption spectrometry (GFAA), inductively coupled plasma atomic emission spectrometry (ICP- AES), or inductively coupled plasma mass spectrometry (ICP-MS).

35.5 Calculations

All elemental concentrations are to be presented in μg/g on a dry weight (moisture-corrected) basis.

Total sulphur (S) concentrations obtained in this analysis should compare favourably (within 10%) with those determined by dry combustion analysis (**TOTAL SULPHUR, NITROGEN & CARBON ANALYSIS PROCEDURE (#17)**).

PROCEDURE #36
EPICUTICULAR WAX STRUCTURE ANALYSIS

36.1 Background 90
36.2 Sample Preparation & Analysis 90

36.1 Background

Epicuticular wax structure may be affected by exposure to air contaminants. The structure analysis is conducted using scanning electron microscopy (SEM).

36.2 Sample Preparation & Analysis

The vials containing the needles are to be warmed to room temperature. Sections (5 mm) from the mid-portion of the needles are to be mounted onto brass stubs using double-sided tape, and coated with gold:palladium (80:20), or equivalent, to a 45 nm thickness. Needle segments are to be evaluated and scored for crystalline epicuticular tube distribution within or adjacent to the stomatal antechamber. Wax tube density for three stomata per needle are to be recorded at 1,300x magnification under a field-emission scanning electron microscope operated at 5 kV accelerating voltage. The first stoma observed that does not manifest particulate, fungi or algae that would interfere with observation of the underlying EW structure is to be selected for evaluation, followed by the next two stomata from the same row of stomata (providing no interference from particulates, fungi or algae). SEM images are to be captured using an energy dispersive analyzer, and then transferred with a digital image recording system. Images are to be processed and analyzed using an Image Processing Tool Kit 4.0 Adobe Photoshop program. Images may be processed with a range of Gaussian blur filters to improve resolution. Threshold and area fractions are to be measured with the global filter.

PROCEDURE #37
EPICUTICULAR WAX COMPOSITION ANALYSIS

37.1 Background **91**
37.2 Reagents: **91**
37.3 QA/QC **91**
37.4 Extraction **92**
37.5 Purification **92**
37.6 GC-MS Analysis **92**
37.7 Calculations **93**

37.1 Background

The components of the epicuticular wax layer may be affected by exposure to air contaminants. The analysis is based on Percy and Baker (1990[24]) and Percy et al. (2009[25]).

Variances to this procedure are permitted, particularly in the instrumentation. Extraction and purification methods may also vary, if required to match to the analytical instrument. All variances are to be reported to the TEEM Program Manager.

37.2 Reagents:

1. Chloroform ($CHCl_3$)
2. Hexane
3. 50:50 Hexane:Chloroform
4. 50:50 Chloroform:Petroleum Ether
5. N,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)

37.3 QA/QC

1. Tetracosane (20 g/mL) as an internal standard.
2. Calibration standard, containing dodecanoic acid, docosane, eicosane, eicosanol, hexacosane, heptacosane, hexadecanoic acid, and octadecanol, at appropriate concentrations.
3. Relative retention time standard of cholesteryl n-octanoate.
4. Replicate (1 per batch): from one randomly selected sample within the batch.

---

[24] Percy KE, Baker EA (1990) Effects of simulated acid rain on epicuticular wax production, morphology, chemical composition and on cuticular membrane thickness in two clones of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) New Phytol.116: 79-87

[25] Percy KE, Manninen S, Häberle K-H, Heerdt C, Werner H, Henderson GW, Matyssek R (2009) Effect of 3 years' free-air exposure to elevated ozone on mature Norway spruce (*Picea abies* (L.) Karst.) needle epicuticular wax physicochemical characteristics. Environ. Pollut. 157:1657-1665

37.4 Extraction

1. Add 10 mL $CHCl_3$ to the vial with the needles, and swirl for 5 sec.
2. Transfer $CHCl_3$ to a glass syringe barrel containing a Supelco (or equivalent) frit, mounted over a pre-weighed collection vial (±10 μg). Allow the sample to pass through the frit under the force of gravity only.
3. Rinse needles, vial and syringe barrel with 1 to 3 mL $CHCl_3$.
4. Evaporate the $CHCl_3$ in a fume hood (no heat or air or nitrogen gas stream).
5. Weigh the glass vials (±10 μg) to determine wax quantity.
6. Oven-dry the needles at 70°C to determine dry weight for wax composition calculations.

37.5 Purification

1. Dissolve wax in 2 mL 50:50 hexane:chloroform
2. Add 25 μL tetracosane internal standard.
3. Run sample through a pre-wetted (hexane) solid-phase extraction (SPE) column.
4. Rinse vial with 1 mL $CHCl_3$, add to top of SPE column.
5. Run, under gravity, 4 mL hexane through the SPE column.
6. Run, under gravity, 4 mL 50:50 hexane:chloroform through the SPE column.
7. Elute the waxes from the SPE column using 6 mL 50:50 chloroform:petroleum ether.
8. Evaporate the eluate under nitrogen, until dry.

37.6 GC-MS Analysis

1. Dissolve wax in 500 μL 50:50 hexane:chloroform.
2. Transfer 100 μL of sample into GC vials.
3. Add 5 μL N,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) through the vial insert.
4. Derivatize for 30 minutes at 70°C.
5. Load 2 μL of sample onto a J&W DB-1HT fused silica capillary column (15 m long; 0.32 mm internal diameter) with procedure silicone liquid-phase (0.25 µm film thickness), or equivalent.
6. Typical GC parameters are:
   a. Helium carrier gas flow of 4.4 mL/min.
   b. 70°C initial injector temperature, increasing to 125°C at 18°C/min., 125 to 395°C at 12°C/min., and remain at 395°C for 15 min.
   c. temperature program of 70 to120°C at 20°C/min., followed by 120 to 390°C at 6°C/min.

   d. 40 min. run time.

7. Typical mass-spectrometer parameters are:
   a. Scan mode, 25 to 800 mass/charge ratio.
   b. 230°C source temperature.
   c. 150°C quad temperature.

Variances in GC and mass spectrometer settings are permitted; all variances are to be recorded and reported with the results to the TEEM Program Manager.

37.7 Calculations

A Varian Workstar (version 4.01) software system is to be used to integrate peak areas and calculate percentage homologue composition (±0001%). Relative retention times (RRT) are to be calculated from the co-injection of cholesteryl n-octanoate. GC assignments are to be made on the basis of appropriate wax homologues.

Epicuticular wax chemical composition is presented as proportion by homologue identified in each wax sample recovered. Wax quantity is to be expressed in units of mg wax/g air dried needle weight.

PROCEDURE #38
PLANT COMMUNITY ASSESSMENT

38.1 Background 94
38.2 Cover Class Assessment.....94
38.3 Daubenmire Summary 95
38.4 Standard Random Walk 97

38.1 Background

This procedure applies only to the vegetation plot at the interior monitoring site.

Canopy cover, frequency of occurrence, and composition by canopy cover are to be evaluated for the ground cover and forb species within each of the 10 small, two medium and the large subplots using the Daubenmire (1959[26]; Coulloudon et al., 1996[27]) method of cover class estimation.

In addition to the quantitative community assessment in the vegetation plot, a species list for the site overall is to be compiled over a 30-min walk through the stand.

38.2 Cover Class Assessment

For consistency within a single monitoring cycle, the assessment is to be performed at all sites by the same qualified vegetation ecologist. An ecologist with substantial taxonomic knowledge and experience in the boreal forest is to conduct this assessment. This assessment is to be conducted in August, when the plants have reached maximum growth for the season.

Observe each subplot from directly above and estimate the cover class for all individuals of a plant species in the subplot, ignoring other species (i.e., estimations are for each plant species separately). Canopies extending over the subplot are to be included in the estimation of cover, even if the plants are not rooted in the subplot. Imagine a line drawn about the leaf tips of the undisturbed canopies (ignoring inflorescence) and project these polygonal images onto the ground. This projection is the “canopy coverage”, expressed as a percentage of the species’

[26] Daubenmire R (1959) A canopy-coverage method of vegetational analysis. Northwest Sci. 33:43-64

[27] Coulloudon B, Eshelman K, Gianola J, Habich N, Hughes L, Johnson C, Pellant M, Podborny P, Rasmussen A, Robles B, Shaver P, Spehar J, Willoughby J (1996) Sampling Vegetation Attributes. Interagency Technical Reference, Cooperative Extension Service, U.S. Department of Agriculture, U.S. Department of the Interior. Revised in 1997 and 1999. Technical Reference 1734-4.

cover within the subplot. From the canopy coverage, determine the appropriate Daubenmire Cover Class, according to the following table.

Daubenmire Cover Classes

Cover Class	Canopy Coverage	Midpoint of Range
1	0 to 5%	2.5%
2	6 to 25%	15.0%
3	26 to 50%	37.5%
4	51 to 75%	62.5%
5	76 to 95%	85.0%
6	96 to 100%	97.5%

The open fields in TEEM Form 08, including the cover class for each subplot, are to be completed as follows:

TEEM Form 08 – Cover Class Assessment

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the data from the last plot tree
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 would be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19	Species	Enter each species present in the subplot on a separate line
20 to 29	Small Subplot No.	Enter the Daubenmire Cover Class for each species in each small subplot
30 & 31	Medium Subplot No.	Enter the Daubenmire Cover Class for each species in each medium subplot
32	Large Subplot	Enter the Daubenmire Cover Class for each species in the large subplot
33	Remarks	Enter observations, difficulties and any other information that may be useful in guiding the interpretation of the data

38.3 Daubenmire Summary

The TEEM Program Manager is to complete the Daubenmire Summary (TEEM Form 09). TEEM Form 09 includes pre-programmed equations for the calculation of Total Canopy, Canopy Cover (%), contribution of each species to overall Species Composition (%), and Frequency (%) of occurrence of each species. Preservation of the equations and linkages among cells in TEEM Form 09 is critical, and therefore, TEEM Form 09 is not a field data collection form. The data to be entered into, and the calculations that are performed within, TEEM Form 09 are as follows:

TEEM Form 09 – Daubenmire Summary

Field(s)	Field Name	Required Information
1 & 2	Page _ of _	Complete after collecting the data from the last plot tree
3 to 7	Site	5-Character site designation
8 to 15	Assessment Date	Date as YYYY-MMM-DD (July 9, 2011 is to be recorded as “2011-07-09”)
16 to 18	Personnel	Three fields are provided for the full names of the personnel involved
19	Species	Enter each species present in the subplot on a separate line
20	#	The number of subplots in which the species occurred at a Cover Class of 1
21	#	The number of subplots in which the species occurred at a Cover Class of 2
22	#	The number of subplots in which the species occurred at a Cover Class of 3
23	#	The number of subplots in which the species occurred at a Cover Class of 4
24	#	The number of subplots in which the species occurred at a Cover Class of 5
25	#	The number of subplots in which the species occurred at a Cover Class of 6
26	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 1 (Field 20) times the midpoint percentage for this cover class (2.5%)
27	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 2 (Field 21) times the midpoint percentage for this cover class (15%)
28	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 3 (Field 22) times the midpoint percentage for this cover class (37.5%)
29	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 4 (Field 23) times the midpoint percentage for this cover class (62.5%)
30	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 5 (Field 24) times the midpoint percentage for this cover class (85%)
31	Product	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The result of the multiplication of the number of subplots containing the species at a Daubenmire Cover Class 6 (Field 25) times the midpoint percentage for this cover class (97.5%)
32	Total Canopy	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The sum of the products (i.e., the sum of Fields 26 to 31)
33	Canopy Cover %	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The percent canopy cover of the species, derived by the division of the Total Canopy (Field 32) by the total number of subplots (10), rounded to the nearest whole number

Field(s)	Field Name	Required Information
34	Canopy Cover Total	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The sum of the Canopy Cover values calculated for each species (i.e., the sum of all Field 33 values)
35	Species Composition %	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The contribution of the Canopy Cover of each species (Field 33) to the total composition of the cover, calculated by dividing each species' Canopy Cover (Field 33) by the Canopy Cover Total (Field 34), multiplied by 100, rounded to the nearest whole number
36	Frequency %	**DO NOT ENTER DATA INTO THIS FIELD.** This is an automated calculation. The number of subplots in which the species occurred, divided by the total number of subplots, multiplied by 100

38.4 Standard Random Walk

In addition to the quantitative cover data collected within each of the vegetation subplots, a "standard random walk" through the site is to be conducted to identify the presence of species not present within the subplots. This is a presence/absence survey only; quantitative cover or abundance data are not required.

The "standard" component of the walk is to be measured in time – the walk is to be conducted for a period of 30 min. The "random" component of the walk relates to the absence of a specific survey pattern or trail that must be followed. The walk is to cover the entire jack pine monitoring site, avoiding stand edges where transition from the jack pine ecological analogue forest type gives way to other vegetation types and soil conditions. The walk must avoid entry into the vegetation and soil plots.

Remarks as to the distribution or number of occurrences of each species should be made. The species list derived on the standard random walk is to be recorded in field note format (a TEEM Form is not provided).