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1   TITLE: NaCl monitoring seawater influence on the growth of *Trametes versicolor*

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15

16   FOOTNOTES TO THE TITLE: Salt effects on biodegradation of Tsunami wood debris

17

21

22   **Abstract**

23   There are only a few scientific data about the function of ecosystems after tsunami

24   disasters. The ecosystems help the environment to recover after a disaster and therefore

25   the research on its function is important. We estimated the seawater influences on wood

26   degradation after tsunami disaster by the growth of *Trametes versicolor*. NaCl in debris

27   was monitored to estimate the impact of tsunami on wood blocks. The debris from the

28　Great East Japan Earthquake on the pacific coast in March 2011 was used for the

29　simulations. Its growth on debris was compared with those on seawater treated woods,

30　and the amount of sodium chloride was examined to know the approximate amount of

31　salts in the samples. As a result, this common white-rot fungus degraded wood debris in

32　the same way as sound sapwood. Although the study was conducted at laboratory level,

33　this is the first report from the real debris, which assessed the fungal decomposition

34　ability of the ecosystem after tsunami disaster.

35

36　**Introduction**

37　　　Tsunami loads have been a huge problem in disaster areas. After the Great East

38　Japan Earthquake on the pacific coast in March 2011, it is estimated that 20,188

39　thousand tons of debris accumulated. Since this type of debris was usually mixed with

40　pollutants like anonymous chemicals and scrap metals (Karube et al. 2015), thus loads

41　of classification and detoxification processes were required before recycling,

42　incineration or landfill disposal for those debris (Ministry of the Environment, 2011).

43　The processes took a time, and consequently most debris remained in disposal stations

44　for ages. As for the Great East Japan Earthquake, in the end, 82 % was recycled, 12 %

45　was incinerated and 6% was landfilled (Ministry of the Environment, 2015). According

46　to the Reconstruction Agency in Japan, which was established after the Great East Japan

47　Earthquake, half of the debris in Miyagi prefecture still remained in the cue for disposal

48   in 2013. About 14% of over two billion tons of the debris from Miyagi and Iwate

49   prefectures was wood debris (Ministry of the Environment, 2015). The treatments of

50   debris from the Great East Japan Earthquake were completed in 2014. The recovery of

51   normal life in these area is the most urgent necessity after the natural disaster (Ministry

52   of the Environment, 2011; Chandrasekhar et al., 2015), and therefore, there has not been

53   many case studies of recoveries of ecosystem in tsunami disaster areas.

54   Here, we evaluated the fungal ability on decomposition of the wood debris

55   from this huge disaster that occurred over a short time span. All analysis with debris

56   was conducted in 2012 just after we received the debris from Miyagi prefecture.

57   *Trametes versicolor* was employed as a model microbe since it is a common white-rot

58   fungus, which attacks a wide range of woods. This fungus occurs commonly in forests

59   and mountain sides but also attacks houses and buildings. It was used for the wood

60   decaying test for industrial purpose (Japanese Industrial Standards JIS_2010). Since

61   after a serious disaster there is no clear single factor which will affect biodegradation

62   efficiency, we studied the influence of salt. We compared the data with that from the

63   samples prepared with artificial seawater based on a NaCl parameter, sodium chloride

64   contents. Salt inhibition on fungal growth is already reported but there is no detailed

65   data after 1970s, and also there is no study using seawater.

66   The information of ecological cycles after tsunami disaster, especially near the

67   urban area, will be helpful to reconstruct the area. In this study, *T. versicolor* attack level

68   on wood debris was measured, and the data was compared with control blocks, which

69   were treated with artificial seawater. The NaCl contents were used to get a comparative

70   parameter. In order to estimate the tested debris condition, the influence of seawater

71   immersion and washing were estimated also using NaCl contents as a parameter. This

72   study provides the novel information about biodegradation of wood debris after tsunami

73   disaster.

74

75   **Materials and methods**

76   *Trametes versicolor*

77   A white-rot fungus, *T. versicolor* (L. ex Fr.) Quel was used for the decay test,

78   which is available in the Deterioration Organism Laboratory (DOL) of the Research

79   Institute for Sustainable Humanosphere, Kyoto University, Japan. *T. versicolor* was pre-

80   cultured in a 100 ml of nutrient solution (4.0 % glucose, 0.3 % peptone, and 1.5 % malt

81   extract) for around a week until the fungus produced granules.

82

83   Wood blocks

84   Standard wood blocks were prepared with sound sapwood of

85   *Cryptomeria japonica* D. Don (20 (R) x 20 (T) x 10 (L) mm).

86   In addition, two groups of seawater treated wood blocks were prepared, group

87   A as a model sample of waterborne debris such as floating driftwood or lumber and

88   group B as a model sample of seawater-washed log after tsunami. In group A, the wood

89   blocks were soaked in 200 ml artificial seawater (Daigo's artificial seawater SP for

90   marine microalgae medium, Nihon pharmaceutical Co. Ltd., Japan) with 100 g quartz

91   sands for 0, 1, 3 and 6 months. Those blocks were air-dried at room temperature for a

92   week before use. In group B, blocks were additionally treated with artificial seawater

93   according with the method of wood surface treatment in JIS K 1571-2010: They were

94   immersed in the seawater for 5 hours at 25 ± 2 °C and dried for 19 hours at 40 ± 2 °C

95   during 10 days before use.

96   Tsunami wood debris arrived from Natori city, Miyagi prefecture in 2012 July

97   (kindly supplied from Dr. K. Kashimura, Chubu University, Japan). They were from a

98   part of the debris in the cue of disposal treatment after the Great East Japan Earthquake

99   of March 11, 2011 for 16 months. Fifteen wood debris were randomly selected and cut

100   into 20 (R) x 20 (T) x 10 (L) mm as same as standard wood blocks (Fig. 1). The

101   condition of the debris did not allow us to identify its wood species, however it seemed

102   that there were at least five or six wood species in the debris. By identification on gross,

103   the 15 debris contained four hardwoods and 11 sapwoods containing *C. japonica*.

104

105   Seawater influence on the growth of *T. versicolor*

106   To learn the decomposition occurrence on waterborne debris, seawater

107   influence on growth of *T. versicolor* was examined with the wood blocks from group A.

108   Salt immersion into the blocks was estimated by the weight change before and after

109   soaking. Experiments were conducted with 12 repetitions for each soaking interval. A

110   monoculture decay test was conducted according to JIS K 1571-2010. All sample blocks

111   were weighed after oven drying at 60 ± 2 °C for 3 days and sterilized with gaseous

112   ethylene oxide prior to the decay test. A glass jar containing 250 g quartz sand (30-50

113   mesh) and 80 ml nutrient solution was autoclaved before use. Three ml of pre-cultured

114   liquid fungus suspension containing granules were inoculated to the glass jar medium

115   and incubated till the mycelia fully covered the surface of quartz sand in the jar. Thus,

116   wood samples were placed on the mycelial mat. They were incubated for 12 weeks at 26

117   ± 2 °C. Three samples were placed in one glass jar. Fungal attack to the wood samples

118   was estimated by comparing the oven-dried masses before and after the test. Hardness

119   was also measured by a durometer (hardness tester: Asker CL – 150H with DD2 tester,

120   Kobunshi keiki Co. Ltd., Japan).

121

122   Debris decay test

123   As for debris, blocks were obtained from both edges of 15 debris because each

124   edge could have different environmental effects such as one edge had been exposed to

125   the air and light but the other side had been buried. Therefore total 30 blocks were used

126   for a decay test. Sound sapwood of *C. japonica* was used as control test samples. For

127   positive control, 5 no-treated blocks were prepared. For negative control, 5 blocks from

128   group B were used. The monoculture decay tests were conducted as described above.

129

130   NaCl contents

131   Salt contents in debris and group B wood blocks were unknown, therefore they

132   were estimated by contents of sodium ion as below. Approximately 0.1 g fractions were

133   collected from each sample block. They were kept in a melting pot and heated at 150 °C

134   for 3 hours in 60 % nitric acid to remove all organic materials. Ash remaining in the

135   melting pot was dissolved in distilled water, whose amount was 100 times volume of the

136   initial fractions. The ahs-containing solutions were filtered (DISMIC-13 cp, 0.20 μm,

137   Advantec, Japan). NaCl contents in the wood samples were estimated by Na content in

138   the digested solution using Inductively Coupled Plasma Optical Emission Spectrometer

139   (ICP spectrometer) (SPS-7800 Plasma Spectrometer, SII - Seiko Instruments Inc.,

140   Japan). A small amount, about 10 cm^3, was injected into the instrument to measure its

141   contents. The standard solutions were made by a commercial sodium standard solution

142   (Na 1000 ppm, Nacalai tesque). To estimate the unpredictable Na contents in the

143   seawater treated wood blocks and debris, standard curves were obtained with 0, 1, 10

144   and 100 ppm standard solutions to cover the wide range of concentration, and the data

145   under 10 ppm Na contents were double-checked with 0, 1 and 10 ppm standard curves.

146   The Na estimation with ICP were conducted on the sample blocks of group B

147   used for decay test and debris. The middle parts of each debris were used to measure the

148   salt contents. 15 debris blocks were used to estimate NaCl contents in the debris.

149

150

151   Statistics

152   Kruskal-Wallis test was used to analyze the decaying rate of debris and controls.

153   On the other hand, the weight loss and hardness change before and after decay test was

154   analyzed with Wilcoxon test. Na contents in the debris were compared with the controls

155   by the Mann-Whitney test. Mann-Whitney test was also applied to examine the

156   hardness change by seawater immersion with each soaking interval. JMP 9.0 software

157   was used for all analyses.

158

159   **Results**

160   Seawater influence on fungal attack of *T. versicolor*

161   This decomposition test with group A blocks was conducted to learn the impact

162   of seawater immersion on biodegradation. Longer immersion in the artificial seawater

163   drove salt accumulation into the wood blocks (Fig. 2A, $p < 0.001$, $X^2 = 40.976$,

164   Kruskal-Wallis test). The weight increase of sample blocks after 1, 3 and 6 months

165   immersion were observed and it indicated that minerals in the artificial seawater

166   penetrated gradually into the wood blocks (Fig. 2A). On the other hand, hardness did

167   not changed by this soaking treatment ($p = 0.485$, $X^2 = 2.446$, Kruskal-Wallis test).

168   *T. versicolor* attack caused a significant weight loss in all samples but not

169   hardness change (Fig. 2BC) (0 month: $p < 0.001$, $X^2 = 17.280$ in weight loss parameter

170   and $p < 0.001$, $X^2 = 11.608$ in hardness parameter, 1 month: $p < 0.001$, $X^2 = 14.533$ in

171   weight loss parameter and $p = 0.002$, $X^2 = 9.545$ in hardness parameter, 3 month: $p <$

172   0.001, $X^2 = 17.295$ in weight loss parameter and $p = 0.644$, $X^2 = 0.213$ in hardness

173   parameter, 6 month: $p = 0.008$, $X^2 = 7.053$ in weight loss parameter and $p = 0.312$, $X^2 =$

174   1.022 in hardness parameter, Mann-Whitney test). The intense fungal attack was

175   observed on the surface of 1 month seawater soaking samples (Supple. 1), but their

176   degradation level was as same as control blocks (Fig. 2B). Thought the considerable

177   weight loss was occurred in all samples, the decay rate decreased with the immersion

178   period ($p < 0.001$, F = 27.580, linear regression analysis), which reflected the hardness

179   change with 3month and 6 month immersion samples (Fig. 2C). It meant that longer

180   immersion in the seawater considerably made *T. versicolor* difficult to attack wood.

181

182   Debris decay test

183   The decay level of tsunami wood debris was compared with naïve (positive

184   control) and group B (negative control) blocks to estimate the potential biodegradation

185   of debris in the nature. *T. versicolor* attacked the debris without any difficulties. The

186   mass loss from the decay test in wood debris was 17.6 ± 2.18 (average ± standard error,

187   SE) wt%, while it was 21.2 ± 1.57 wt% in no-treated sample blocks. Hardness value

188   decreased by fungus attack ($p < 0.001$, $X^2 = 15.196$, Wilcoxon test). There was no

189   significant difference in decaying levels among wood debris, no-treated and seawater

190   surface-treated wood blocks ($p = 0.194$, $X^2 = 3.282$, Kruskal-Wallis test). At the initial

191   stage of incubation, group B wood blocks were less attacked (Supple. 2) however the

192   these blocks even got slightly more attack after 12 weeks incubation (Mass loss, 26.5 ±

193   2.17 wt%).

194

195   NaCl contents in the debris

196   To obtain the comparative parameter, NaCl contents in the debris and group B

197   samples were measured by the detection of sodium ion. Na contents were significantly

198   low from the negative controls ($p = 0.001$, $X^2 = 10.714$, Mann-Whitney test). The debris

199   samples contained 0.12 – 3.3 mg / g (average: 1.14 mg / g) Na and the group B blocks

200   contained 15.17 – 25.71 mg / g (average: 19.32 mg / g). Na contents in non-treated

201   wood blocks were below the measurable limit. The results were similar with 0, 1 and 10

202   ppm standard curve.

203

204   **Discussions**

205   This study demonstrated the salt effects on the growth of *T. versicolor*. The real

206   debris from the Great East Japan Earthquake was also analyzed. The inhibition on the

207   fungal growth by seawater was observed, however the results indicated that debris

208   certainly contained salt, but not at the critical level. According to the data from ICP

209   spectrometer, the debris contained average 1.14 mg/g Na and they were attacked by the

210   white-rod wood decaying fungi as same as the control sound sapwood.

211   It is well known that salt has a function to inhibit the fungal growth (Tresner

212   and Hayes 1971; Sato et al. 2014), on the other hand, there are few reports of tsunami

213   influence on fungal growth the nature. Seawater normally has 3.5 % salt content. Our

214   experiments demonstrated that wood blocks could accumulate around 5 % salt by 3

215   month if they were soaked into seawater. The growth rate of *T. versicolor* was

216   significantly delayed if the wood blocks contained this amount of salt (Fig. 2, Supple. 3).

217   Seawater contains many ions, but NaCl is one of the representative component of

218   seawater. The results matched with the former study using 29 *T. versicolor* strains,

219   which added sodium chloride into media (Tresner and Hayes 1971). Moreover the

220   hardness increase of wood blocks with the salt contents was observed in this study. It

221   would be amenable with the fact that NaCl treatment improves tensile strength of

222   fiberboard (Uraki et al. 2005). NaCl is also known to change the water movement inside

223   of woods (Yang et al, 2011). It seemed, however, that biodegradation process would not

224   be really disturbed by salt after a real disaster. This is probably because that fungal

225   tolerance to NaCl is varied in wide range (Tresner and Hayes 1971). It is highly possible

226   that salt-sensitive microbes simply do not colonize on the debris after tsunami disaster

227   (Higashijima et al. 2012). Actually Sato et al. (2014) isolated the fungi, which were

228   tolerant to 15-20 % salt from the paper objects in Tohoku regions at 5 months after the

229   Great East Japan Earthquake. Debris were often left uncontrolled in the nature after

230   Tsunami disaster. Our samples, which were placed in debris dump for 16 months,

231   contained about 0.001 % Na, which indicated that it contained 0.003 % salt (calculated

232   from NaCl / Na proportion). This would be partly because of wash-off effect of rain

233   (Pérez-Rodríguez et al. 2015; Konoplev et al. 1996; Nasrabadi et al. 2011). The wash-

234   off function of fresh water was successfully confirmed with seawater treated wood

235   blocks (Supple. 4), and we think that same washing-off procedure occurred on the

236   debris due to rain.

237   In the test, the debris was simply classified and numbered by a chunk. Most of

238   them were materials from residential constructions. The 15 debris seemed to contain

239   four hardwood and 11 sapwood containing one *C. japonica*. This variation made

240   difficult to assess the quality of these debris for their different densities and unknown

241   wood preservation treatment before the disaster. Therefore we simply compared the data

242   with well-studied sapwood *C. japonica* as an implementing option to evaluate the debris

243   condition. Sapwood generally has lower natural durability and it is used as a control

244   wood in the decaying test of JIS K 1571-2010. Even with careful consideration about

245   this alternative, our results supported the sufficient ability of microbe to decompose the

246   waterborne organic debris after Tsunami disaster. Decaying scale would be much larger

247   in the nature than this experiments, however it should not be a matter because our

248   additional test with larger wood blocks still supported this conclusion that debris would

249   be decomposed by microbes as same as normal logs in the nature (Supple. 5).

250   Ecological services for human life have been highlighted nowadays and we

251   have just realized that research on its beneficial and detrimental effects is important (Li

252   et al., 2014). Nevertheless ecosystem service has both useful and difficult aspects for us

253   and though it is not easy to control, it can greatly help the recovery process after a

254   tsunami disaster (Costanza et al. 1997; Gómez-Baggethun and Barton 2013; Jansson

255   2013). Moreover, the knowledge of the ecological condition after the disaster will be

256   important for encouraging victims to resume their normal life. Toyofuku et al. (2014)

257   reported that the biotic resilience in Tohoku area was quicker than they had expected

258   and it means that there would be active interactions between living organisms and

259   debris. These debris will stay in nature for a certain time. Generally metal and plastic

260   debris would have a slower impact than organic materials even though they can cause a

261   serious environmental problem. In this study, we examined the wood debris but we must

262   pay high attention also on the metal debris, especially on heavy metals. We must find

263   the way to use well the ecosystem and nature to deal with a natural disaster in this

264   modern world.

265   Natural disaster causes more and more serious problems nowadays because of

266   the urbanization. Many difficulties exceed human efforts. Eventually we can not help

267   relying on ecological purification systems to get back the healthy environment after

268   disasters. When the scale of natural disaster is larger than our assumption, even nature

269   itself needs a long time for the recovery. Therefore assessments after natural disaster in

270   the city area are essential not only for the human habitability but also for the

271   sustainability of natural eco-systems. For the prompt recovery of nature after tsunami

272   disaster, further study on ecosystems is essential.

273

281

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336

337   **Figure legends**

338   Fig. 1 Tsunami wood debris from Natori city, Miyagi prefecture, Japan in 2012 July.

339   A: Arrived debris. Radioactivity was at natural level. B: 15 debris samples. C: 20 (R) x

340   20 (T) x 10 (L) mm sample block used for a decay test.

341

342   Fig. 2 Seawater influence on fungal attack of *T. versicolor.*

343   A: Salt (wt%) absorbed into the wood blocks by the immersion treatment before using

344   for decay test (n = 12) is illustrated with □ and solid line. NaCl contents in the debris,

345   which was calculated with ICP-indicated Na contents by NaCl molecular weight, before

346   using for decay test is shown with ◆ (n = 30). B: The mass loss by decaying test (wt%)

347   on *C. japonica* wood blocks immersed in the seawater for 0, 1, 3, and 6 months (n = 12).

348   The data from debris is shown with gray column (n = 30). C: Hardness change of *C.*

349   *japonica* wood block conditions after decay test. The hardness data from debris is

350   illustrated with gray column for the data before decay test and with dotted column after

351   decay test (n = 28). Vertical bar indicates standard error. Letters at the top of the column

352   indicate the results of the Tukey-Kramer HSD test.

353

Supplemental data

Supple. 1 Photos of the decay test with 1 month seawater immersed samples to see *T.*

versicolor attack. A: Fungal attack on wood block at 1 week after inoculation. Right jar

was for control and left jar was for test. B: Surface of control wood block after 12 week

fungus attack. C: Surface of 1 month seawater soaked wood block after 12 week fungus

attack.

Supple. 2 Decay test with *T. versicolor.*

T. versicolor attacked A: sound sapwood with no treatment (positive control), B: sound

sapwood treated with artificial seawater (negative control), C: wood debris from

tsunami disaster (test). Scale bar at the bottom of the photos indicated 2 cm.

Supple. 3 NaCl influence on growth of *T. versicolor.*

The standard data for the comparison between debris decaying rate and control

wood decaying rate based on sodium chloride contents.

A: Fungus growth (diameter) in 90 mm petri dishes on PDA medium contained 0 %

NaCl: ○, 1 % NaCl: □ 3 % NaCl: ◇, 5 % NaCl: ● 10 % NaCl: ■ Vertical bar indicates

standard error. B: Photo of fungus growth at 3 month after inoculation.

372   To get the simplest standard of salt effects on the growth of *T. versicolor,* NaCl

373   influence on growth of *T. versicolor* was observed on a medium. Potato dextrose agar

374   (PDA: potato extract, 0.4 %; glucose, 2.0 %; agar, 1.5 %) contained 0, 1, 3, 5 and 10 %

375   NaCl was prepared in 90 mm petri dishes and a pre-cultured 8 mm disk of *T. versicolor*

376   was placed in the middle of each petri dish. Fungal growth was measured for 12 weeks

377   at 26 ± 2 °C. Experiments were conducted with 10 repetitions for each NaCl

378   concentration.

379   As it is well known that the NaCl added to PDA affected negatively on *T.*

380   *versicolor* growth (Supple. 4A) ($p < 0.001$, $F = 47.808$ in the parameter of growth speed

381   difference along with NaCl contents, linear regression analysis). It took 7 days, 3 weeks

382   and 2 months for the mycelia of *T. versicolor* to cover the media with 0, 1 and 3 % NaCl

383   contained media respectively. Approximately 67.5 % surface of medium was covered by

384   mycelia at three month interval with 5 % NaCl contained PDA, and no growth was

385   observed with 10 % NaCl contained PDA (Supple. 4B).

386

387   Supple. 4 NaCl loss test by washing to simulate the function of rain.

388   The wash-off effect by rain had been examined to monitor the conditional

389   influence of the debris. A: washing condition with the indication of fractions obtained

390   parts. B: Salt reduction rate in wood blocks after washing at 7th day (n = 5). Salt

391   quantities were estimated with Na contents detected by ICP spectrometer (SPS-7800

392   Plasma Spectrometer, SII - Seiko Instruments Inc., Japan). Vertical bar indicates

393   standard error. Letters at the top of the column indicate the results of the Tukey-Kramer

394   HSD test.

395   Samples treated with artificial seawater were used to simulate the washing function on

396   salt reduction by rain. Wood sample blocks were placed on the plastic mesh so that all

397   water was running away from a test arena. Those blocks were showered with 10 ml, 100

398   ml and 500 ml distilled water every day for 7 days. Fractions were obtained at 7th day

399   and they were treated with nitric acid, and then adjusted with distilled water. Salt

400   residue in the wood samples were estimated by Na content in the digested solution

401   using ICP spectrometer. A small amount, about 10 cm^3, was injected into the instrument

402   to measure its contents of Na. Standard carve was obtained with 0, 1, 10 and 100 ppm

403   standard solution adjusted by a commercial sodium standard solution (Na 1000 ppm,

404   Nacalai tesque). Some of detected values exceeded 100 ppm, however the data

405   demonstrated that a water shower had a certain effect on washing out NaCl from the

406   wood blocks ($p < 0.001$, F = 12.100, linear regression analysis). This wash-off effect by

407   rain had been examined to monitor the conditional influence of the debris.

408

409   Supple. 5 Mass loss from the larger scale decay test with 20(R) x 20(T) x 110(L) mm

410   wood block (n=5).

411   Debris no. 1-5 were used for tests and control samples were made with sound sapwood

412   *C. japonica*. Decay level was same in control and test samples (p = 0.502819, T test).

413

414
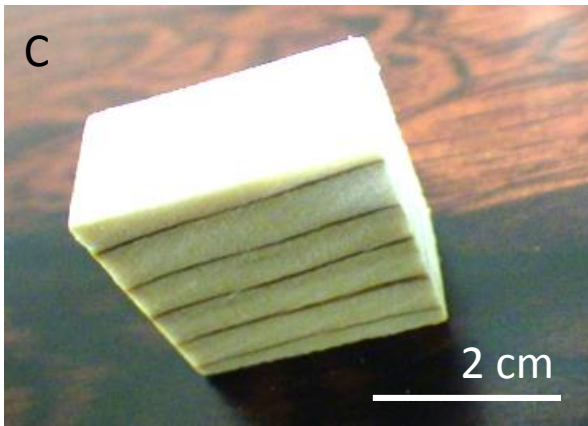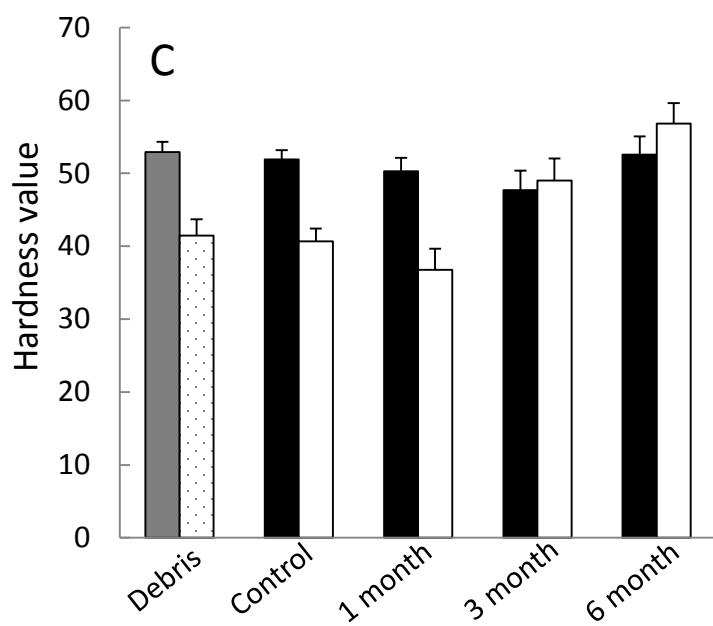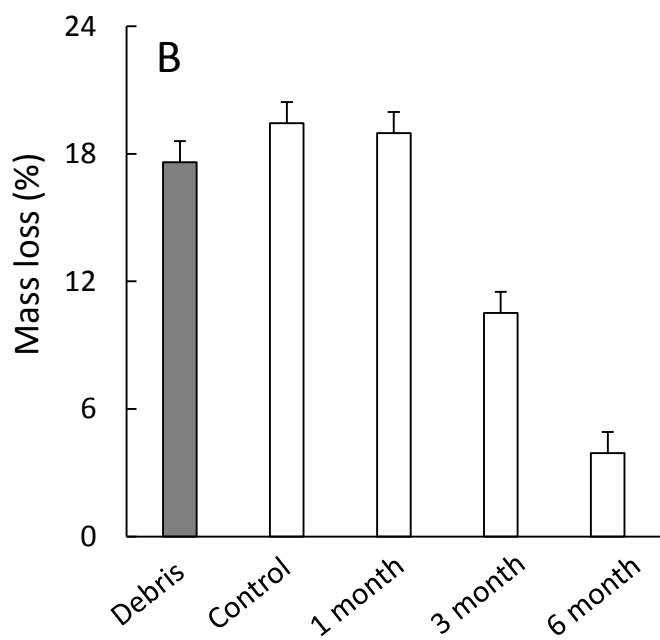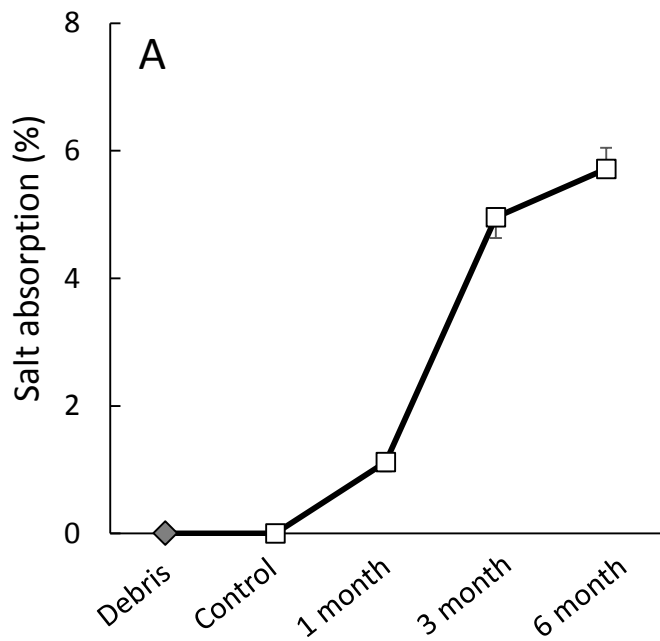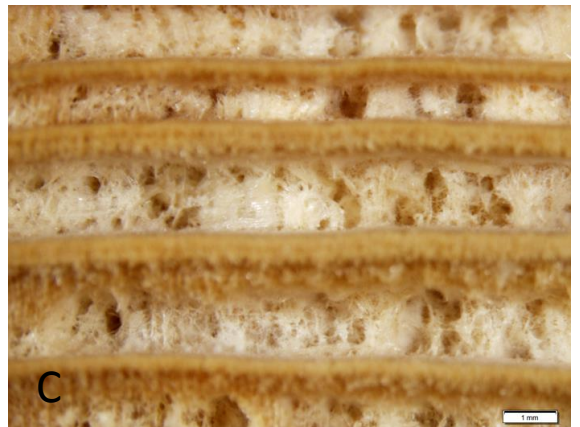
415

Fig 1

Fig 2
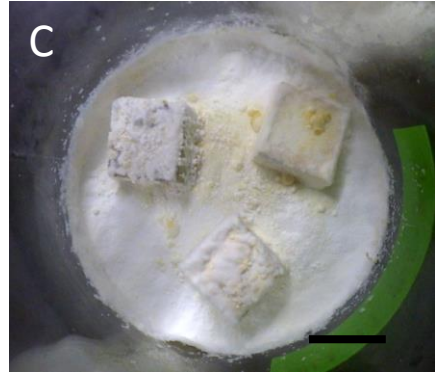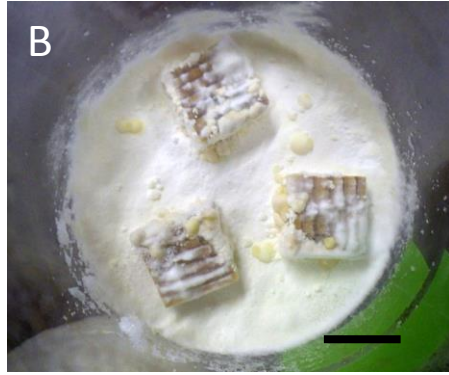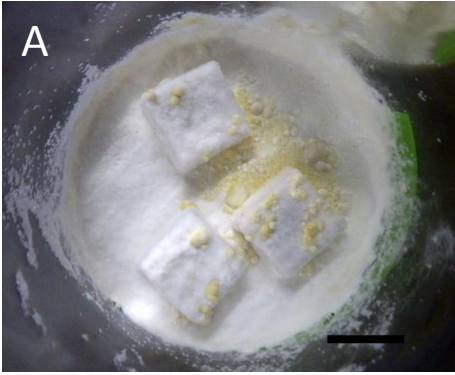
Supple 1
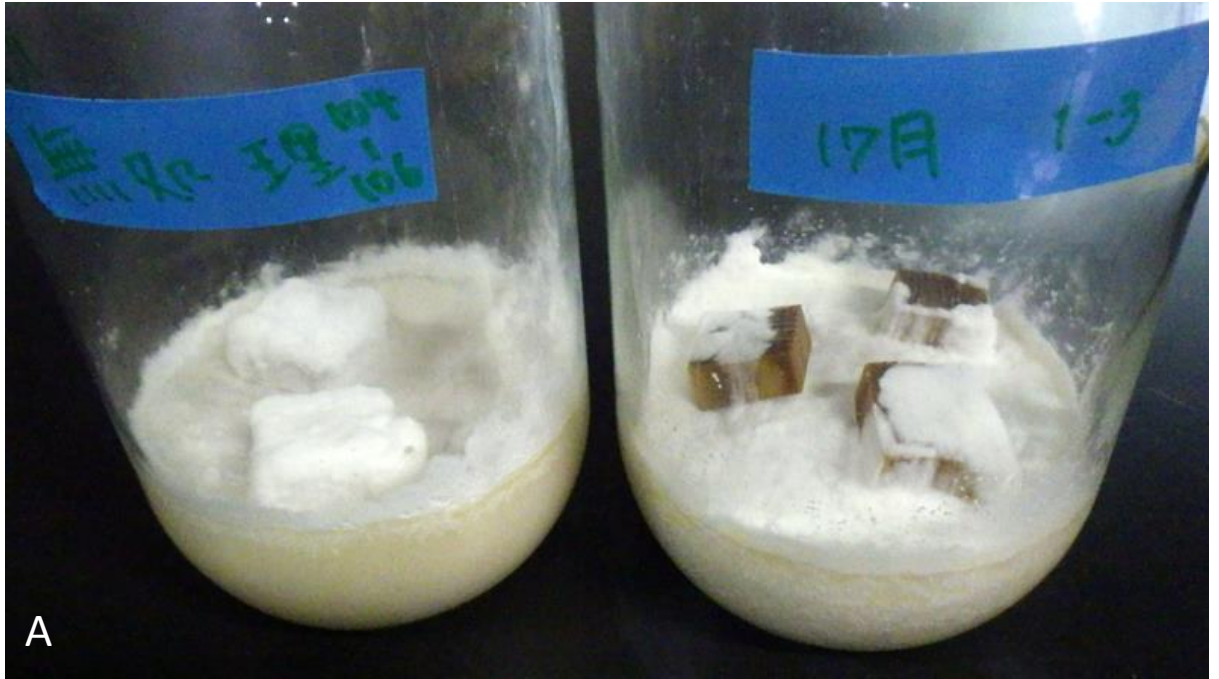
Supple 2

Mass loss(%) of middle sized wood blocks (n = 5)		
	Control	Debris
% (wt)	8.00	5.60
SE	0.015	0.031

Supple 3

A

B

Supple 4

A

1 cm

B

NaCl reduction rate

1

0.8

0.6

0.4

0.2

0

a

ab

bc

c

0 ml   10 ml   100 ml   500 ml

Water quantity for washing/day

Supple 5